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Journal de l'office des recherches sur les pêcheries du Canada

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Editorial[1]

Over the last few months it has become apparent that the *Journal* is facing a crisis which we can solve only with the understanding and cooperation of our contributors. Rising costs of producing the *Journal*, combined with a rate of submissions that is increasing exponentially, are seriously straining our resources. The recent increase in page charges (from $30 to $40 per page — mandatory for all submissions) covers only a fraction of the higher production costs. A backlog of manuscripts is accumulating but we hope to meet existing commitments through expanded *Journal* issues for November 1976 through March 1977.

Nevertheless, we cannot continue to accommodate the number of manuscripts we receive. Recent federal cutbacks, salary increases, and a high rate of inflation in the printing industry are forcing us to try to limit the quantity and size of manuscripts submitted. We hope to accomplish this by further increasing our standards of acceptance, and by communicating to potential authors through this editorial an elaboration of the more significant aspects of our editorial policy.

Basing the acceptance or rejection of manuscripts on the extent and significance of new knowledge presented has always been our policy; this will continue, but in future must be applied more stringently. A significant proportion of data generated in aquatic science is necessarily purely descriptive. These data are basic to the advancement of science, but cannot be accommodated in primary research journals. The report literature was created specifically for this type of work. The function of the primary journal is to communicate concrete advances in knowledge or theory; widely applicable principles and methodology; and critical, constructive appraisals of broad areas of research. In other words, we deal in synthesis and interpretation rather than in pure facts and description.

Many manuscripts we receive are descriptive, and it would relieve part of our editorial burden if these were submitted to more appropriate journals. We refer particularly to work loosely termed "case history studies." Common topics include patterns of age and growth in specific fish populations; the effects of toxicant A on organism B, or of effluent C on community D; environmental baseline studies; and range extensions that have little zoogeographical significance. We do not denigrate such work, but if no new insight or scientific principle is reported the *Journal* will not publish it.

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[1] Reprinted from Volume 33, No. 12, p. 2697–2698.

We continue to welcome original, interpretive research articles and notes, reviews, perspectives, and letters to the editor, but we reserve the right to return manuscripts that obviously do not satisfy our requirements without resorting to the normal reviewing process. Also, we may be forced to refer some well-qualified papers to other journals if the submission rate does not drop.

Authors can do much to present their work as concisely as possible. Overly long articles and notes will be edited vigorously in order to maximize the number of items per volume. We will continue to encourage definitive studies by discouraging any apparent duplicate or fragmentary publications. With the continuing cooperation between authors, reviewers, and editors, we hope that the *Journal* will overcome this crisis and maintain its high standards of scientific communication.

J. C. STEVENSON
Editor and Director of Scientific Information

Éditorial[1]

Il est devenu évident au cours des quelques derniers mois que le *Journal* est en face d'une crise que seules la compréhension et la coopération de nos collaborateurs nous permettront de résoudre. Des coûts de production à la hausse combinés à un nombre d'articles reçus qui augmente à un rythme exponentiel taxent sévèrement nos ressources. La récente augmentation du taux par page (de $30 à $40 la page—applicable à tous les articles) ne couvre qu'une fraction de l'augmentation des coûts de production. Il s'accumule un arriéré de manuscrits, mais nous espérons pouvoir respecter nos engagements présents en augmentant le volume des numéros du *Journal* de novembre 1976 à mars 1977 inclusivement.

Néanmoins, nous ne pouvons continuer de publier tous les manuscrits que nous recevons. Les récentes coupures budgétaires au fédéral, les augmentations de salaires et le taux élevé d'inflation dans l'imprimerie nous forcent à limiter la quantité et le volume des manuscrits qui nous sont soumis. Nous espérons pouvoir réaliser cet objectif en adoptant des normes d'acceptation encore plus strictes et en exposant aux auteurs éventuels, par le biais du présent éditorial, les aspects les plus importants de notre politique de rédaction.

Notre politique a toujours été d'accepter ou de rejeter un manuscrit selon l'étendue et l'importance des connaissances nouvelles qu'il contient; nous poursuivrons cette politique, mais à l'avenir il nous faudra l'appliquer plus strictement. Une bonne proportion des données qu'engendre la science aquatique sont nécessairement purement descriptives. Ces donnés sont fondamentales à l'avancement de la science, mais ne peuvent trouver place dans des périodiques de recherche de fonds. Des publications ont été créées spécifiquement pour accomoder les rapports sur ce genre de travail. Le rôle d'un périodique de fonds est de communiquer les progrès concrets de la connaissance ou de la théorie; les principes et la méthodologie d'application générale; et les jugements critiques et constructifs dans de vastes segments de la recherche. En d'autres mots, nous nous occupons de synthèse et d'interprétation plutôt que de faits purs et de descriptions.

Un bon nombre des manuscrits que nous recevons sont descriptifs, et si on les soummettait à des périodiques plus appropriés, notre fardeau éditorial serait allégé d'autant. Nous visons particulièrement les travaux vaguement désignés comme «observations de cas particuliers». Sous cette rubrique, on inclut communément l'âge et la croissance dans des populations spécifiques de poissons; les effets du toxique A sur l'organisme B, ou de l'effluent C sur la communauté D; des études de référence fondamentale sur l'environnement; et des extensions d'aire qui n'ont pas beaucoup de signification zoogéographique. Nous ne voulons pas minimiser l'importance de tels travaux, mais si un manuscrit ne contient pas de vues ou de principes scientifiques nouveaux, le *Journal* ne le publiera pas.

Les notes et articles scientifiques originaux et interprétatifs, les revues, les essais et les lettres au rédacteur continueront d'être les bienvenus, mais nous nous réservons le droit de retourner les manuscrits qui ne sont pas à la hauteur de nos normes et ne peuvent y satisfaire sans qu'on ait recours au processus normal de révision. En outre, il se peut que nous soyons forcé de transmettre à d'autres périodiques des articles qui se qualifient par ailleurs, si le nombre de manuscrits qui nous sont soumis ne diminue pas.

Les auteurs peuvent nous aider beaucoup en présentant leurs travaux de façon aussi concise que possible. Les notes et articles trop longs seront rigoureusement édités, afin de pouvoir publier un nombre maximum d'item par numéro. Nous continuerons d'encourager

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[1] Réimprimé à partir du volume 33, n° 12, p. 2697–2698.

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la publication d'études définitives en décourageant celle d'études fragmentaires et d'études où il semble y avoir duplication. Que les auteurs, les réviseurs et la rédaction continuent de coopérer, et nous espérons que le *Journal* pourra surmonter cette crise et maintenir sa haute qualité de communication scientifique.

J. C. STEVENSON
Rédacteur et directeur de l'information scientifique

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Uptake from Sea Water and Clearance of ^{14}C-*p,p'*-DDT by the Marine Copepod *Calanus finmarchicus*[1]

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Harding, G. C. H., and W. P. Vass. 1977. Uptake from sea water and clearance of ^{14}C-*p,p'*-DDT by the marine copepod *Calanus finmarchicus*. J. Fish. Res. Board Can. 34: 177–182.

It is impossible to measure the uptake rate of *p,p'*-DDT by copepods from sea water directly since uptake and depuration occur simultaneously. Copepods were found to lose *p,p'*-DDT exponentially in flowing sea water with a rate constant of $k_j = 0.048$/day; $\frac{dC}{dt} = -k_jC$. Five experiments were performed to obtain uptake rates with DDT concentrations ranging from 32.5 to 1337 pptr in sea water. Copepod uptake of *p,p'*-DDT from sea water is amply described by a single-compartment model, $\frac{dC}{dt} = k_i \frac{\mu}{\alpha} W - k_jC$, which accounts for influx and efflux of DDT. Calculated influx rate constants, k_i, varied between 1.5 and 2.7×10^3/day. These results enable prediction of DDT levels in nonfeeding copepods if the concentration of DDT in sea water is known.

Harding, G. C. H., and W. P. Vass. 1977. Uptake from sea water and clearance of ^{14}C-*p,p'*-DDT by the marine copepod *Calanus finmarchicus*. J. Fish. Res. Board Can. 34: 177–182.

Il est impossible de mesurer directement le taux auquel les copépodes assimilent le *p,p'*-DDT de l'eau de mer, car la dépuration des copépodes se produit simultanément. Nous constatons que les copépodes perdent le *p,p'*-DDT à un taux exponentiel dans de l'eau de mer courante avec constante de taux de $k_j = 0.048$/jour; $\frac{dC}{dt} = -k_jC$. Nous avons fait cinq expériences en vue de déterminer les taux d'assimilation avec des concentrations de DDT allant de 32.5 à 1337 ppb dans de l'eau de mer. L'assimilation de *p,p'*-DDT de l'eau de mer par les copépodes est amplement décrite par un modèle à compartiment unique, $\frac{dC}{dt} = k_i \frac{\mu}{\alpha} W - k_jC$, qui explique à

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[1] Bedford Institute of Oceanography Contribution.

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la fois l'entrée et la sortie du DDT. Nous constatons que les constantes de taux d'entrée calculées, k_1, sont du même ordre de grandeur, 1.5 à 2.7 × 10^3/jour. Ces résultats permettent de prédire les niveaux de DDT dans des copépodes qui ne se nourrissent pas, si l'on connaît la concentration de DDT dans l'eau de mer.

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IT is well known that DDT has attained global distribution in marine organisms; however, the dynamics of its transfer in the biosphere either from sea water or ingesta is not clearly understood. This paper is concerned with the uptake of ^{14}C-*p,p'*-DDT (2,2-bis-*p*-chlorophenyl-1,1,1-trichloroethane) from sea water at five concentrations by a calanoid copepod and its clearance rate into uncontaminated sea water. The data are described by a simple exponential model that enables prediction of *p,p'*-DDT levels in marine copepods assuming only direct uptake from sea water of known concentration.

Materials and Methods

Zooplankton were collected between August 1974 and April 1975 with a 1-m diam, 752 μm mesh net towed at about 0.5 m/s at the mouth of St. Margaret's Bay, N.S. The codend of the net was fitted with a 2-ℓ glass container to minimize injury to the organisms by water flow. *Calanus finmarchicus* Gunnerus were sorted and maintained in an incubator under low light intensity at 5–6 C as described previously (Darrow and Harding 1975).

Batches of 50 copepods were held for 1 day in about 1800 ml sea water to clear their digestive tracts and to facilitate the removal of net-damaged individuals. (Unless specified, sea water was filtered twice through GFC filters which removed 100% of particles larger than 5 μm [Sheldon and Sutcliffe 1969].) Sea water (1300 ml), contained in 2-ℓ glass-stoppered flasks fitted with ground glass discs and Teflon stopcocks, was spiked with ^{14}C-*p,p'*-DDT (specific activity 6.25 mCi/mM; New England Nuclear Corp.) in small carrier volumes of acetone (<4 μl). The sea water was stirred magnetically at room temperature for 1 h and kept overnight in the incubator at 5–6 C before use. This was sufficient time for the *p,p'*-DDT to equilibrate between the container and sea water. In the uptake series of experiments, batches of 50 healthy copepods were transferred to the spiked flasks in 200 ml sea water. Control flasks contained copepods in 1500 ml sea water. Carrier volumes of acetone had no effect on the viability of the copepods. The sea water in experimental flasks was changed daily to maintain approximately constant *p,p'*-DDT concentrations. Five uptake experiments were performed in this manner. Copepodids IV and V were used for uptake experiments at concentrations of 355 ± 8 (mean ± SE) (August 1974) and 36.3 ± 2.3 ng *p,p'*-DDT/ℓ (April and May 1975). When adult female *Calanus* were abundant in the plankton, uptake experiments were run at 1337 ± 30 (January 1975), 324 ± 13 (February 1975) and 32.5 ± 0.7 ng *p,p'*-DDT/ℓ (March and April 1975). Uptake series were terminated before 20 days since mortality, attributed to starvation (see clearance experiment) and confinement in a small vessel, had reached 40% in uptake and control flasks.

In the 1337 ± 30 pptr series it was decided to terminate the experiment after 6 days because of the erratic behavior and reduced mobility of the copepods. These are well-documented symptoms of DDT poisoning in crustaceans (see Sheridan 1975). All dead copepods were discarded daily. Darrow and Harding (1975) have shown that *Calanus* does not metabolize *p,p'*-DDT over an 8-wk period.

In the clearance studies, batches of 50 copepods containing 15.42 ± 1.06 ppm dry wt *p,p'*-DDT (March 1975) were placed in flowing unfiltered seawater tanks within 505 μm mesh cages of 3725 cm^3 at 2–4°C. Copepods fed on incoming particulates as demonstrated by the fact that guts were green, and they appeared healthy although mortality reached 40% after 39 days when the last batches were analyzed. No *p,p'*-DDT was detected in flowing seawater tanks (<1 pptr) or in control copepods held within the same tanks.

Residues of *p,p'*-DDT and related compounds were extracted from sea water, flasks, and copepods, separately. The contents of each flask were passed through a stainless steel screen in a glass filter holder into a 6-ℓ separatory funnel. Empty flasks were extracted for *p,p'*-DDT in uptake experiments with two rinses of 100 ml acetone and one rinse of 100 ml 15% diethyl ether–hexane (vol/vol), and from air-dried mesh cages in clearance experiments with four rinses of 50 ml 15% diethyl ether–hexane. All solvents were of reagent grade and distilled from glass before use. Sea water was extracted by four, 3-min shakes with 100 ml 15% diethyl ether–hexane and combined with solvent rinses of the filter holder. Total recoveries of *p,p'*-DDT in seawater flasks without copepods were 92.1 ± 1.7% ($N = 12$). No correction is made in the text.

Copepods were removed from the screen by the antennules with fine forceps to avoid rupturing the cephalothorax and placed in a 10-ml glass tissue grinder with about 5 ml sea water. The sea water containing copepods was injected with ^{3}H-*p,p'*-DDT (New England Nuclear Corp.) in 80–100 μl acetone for internal standard recovery determinations; a piece of GFC filter increased grinding efficiency. Following homogenization, tubes were centrifuged for 30 min at $1.4 \times 10^3 g$. The floating lipids and the particulate copepod material remaining in the sea water were extracted separately with 15% diethyl ether–hexane. This solvent system was found to be as efficient for extracting copepod lipids as chloroform–methanol in the method of Folch et al. (1957).

Acetone extractions of container glassware were reduced to a small volume several times by rotary evaporation at 30°C, taken up in hexane twice, and dried over anhydrous sodium sulphate. The diethyl ether–hexane extracts were passed through a sodium sulphate column, similarly reduced to a small volume, and brought to a final volume of 1 ml under nitrogen. Aliquots of 0.5 ml were transferred to scintillation vials and dissolved in 15 ml of Permafluor® (40 ml diluted to 1 ℓ in toluene, Packard Instrument Co.). Double-label liquid scintillation counting was performed on a Packard Tri Carb model 3375 instrument fitted with automatic external standardization to give <2.5% SD for each sample. Extraction efficiencies based on ^{3}H-*p,p′*-DDT recovery were $91.0 \pm 0.5\%$ ($N = 100$). The ^{14}C-*p,p′*-DDT content of each copepod sample was corrected to a 100% recovery.

With the above technique $62.0 \pm 2.5\%$ ($N = 11$) of the total extractable copepod lipid floated to the surface; the balance was centrifuged down with tissue and exoskeleton debris. This percentage was remarkably constant throughout the winter even though the total lipid content of the copepods declined from 42.2 to 4.2% on a dry weight basis. Total *p,p′*-DDT in the lipid compartment was estimated by scaling up the concentration in the "floating" lipid to 100%. The remaining *p,p′*-DDT was assumed not to be associated with lipid, but to be bound to the tissues and exoskeleton.

A right circular cylindrical shape was assumed to approximate the external surface area of a copepod, with a height equal to total copepod length and radius equal to half the broadest section of the cephalothorax. The sea water in contact with this surface at any instant was taken to be 2×10^{-10} m thick.

Results and Discussion

The accumulation of *p,p′*-DDT from sea water by copepods (Fig. 1) could be fitted with linear equations but this would imply that there is no loss from the copepods. An exponential model was adopted (Ruzic 1972; Sheppard 1962) making the assumption that the rates of uptake or clearance are proportional to the actual *p,p′*-DDT concentration in sea water (W) and in/on copepods (C), respectively. A single-compartment model was found to be sufficient for predictive purposes through logarithmic analyses of the data. This contention is supported by the experimentally measured lipid compartment which contains a fairly constant percentage of total copepod *p,p′*-DDT from 10 s to 20 days (Tables 1–3).

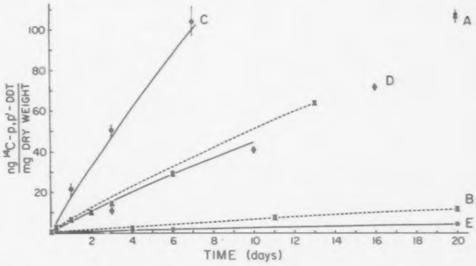

FIG. 1. Uptake of ^{14}C-*p,p′*-DDT from sea water by *Calanus finmarchicus*: A, Stage IV and V copepodids at 355 ± 8ng/ℓ; B, stage IV and V copepodids at 36.3 ± 2.3 ng/ℓ; C, Adult females at 1337 ± 30 ng/ℓ; D, Adult females at 324 ± 13 ng/ℓ; E, Adult females at 32.5 ± 0.7 ng/ℓ.

Since the *p,p′*-DDT concentration in sea water (W) was approximately constant (Tables 1–3), the rate of accumulation of *p,p′*-DDT on and into the copepods can be expressed as,

$$\frac{dC}{dt} = k_i \frac{\mu}{\alpha} W - k_j C \tag{1}$$

where k_i and k_j are the rate constants in and out of the copepods, α is the dry weight of a copepod, and μ is the weight of sea water in contact with a copepod during an infinitesimal time interval. For the uptake experiment, the solution of equation (1) is,

$$C = \frac{k_i \mu W}{k_j \alpha} [1 - e^{-k_j t}] \tag{2}$$

For the loss experiment with W assumed to be zero, equation (1) becomes,

$$\frac{dC}{dt} = -k_j C \tag{3}$$

and

$$C = C_0 \, e^{-k_j(t - t_0)} \tag{4}$$

where C_0 and t_0 correspond to the beginning of the loss experiment.

The clearance of *p,p′*-DDT from adult female *Calanus finmarchicus* is best fitted to a negative exponential curve, $C = 15.42 \; e^{-0.048 \, (t - t_0)}$, $r = 0.96$ (Fig. 2). Using k_j equal to 0.048, uptake data can be fitted to equation (2) after transformation to logarithms,

$$\ln \left[\frac{KW}{KW - C} \right] = k_j t$$

where

$$K = k_i \mu / k_j \alpha$$

TABLE 1. Uptake of ^{14}C-*p,p′*-DDT by *Calanus finmarchicus* (IV's and V's) from sea water of 355 ± 8 ng)/ℓ.

	DDT concentration in/on copepod				
	Lipid				
Time	(*ppm*)[a]	(%)	Total (*ppm*)[a]	Sea water (*pptr*)[b]	No. copepods analyzed/ container
10–11 s	0.17±0.01[c]	45.3±1.0	0.36±0.02	511	49±1
1 h	0.41±0.16	54.8±8.9	0.72±0.21	501±23	50±1
6 h	1.10±0.21	43.3±5.0	2.49±0.22	412±19	44±7
1 day	4.75±1.09	73.0±15.7	6.52±0.40	346±29	47±2
2 days	6.04±0.20	59.7±5.6	10.25±0.36	331±25	51±1
3 days	9.21±0.05	62.1±1.9	14.83±0.38	365±5	47±1
6 days	23.95±3.88	81.3±13.7	29.54±0.87	362±11	46±1
13 days	48.23±5.77	75.4±8.3	63.84±0.82	370±8	33±3
20 days	75.11±3.54	70.0±2.3	107.30±3.34	511±20	28±1

[a] Nanograms ^{14}C-*p,p′*-DDT per milligram dry weight *Calanus*.
[b] Nanograms ^{14}C-*p,p′*-DDT per litre sea water.
[c] Mean ± SE of three containers.

TABLE 2. Uptake of ^{14}C-*p,p′*-DDT by *Calanus finmarchicus* (IV's and V's) from sea water of 36.3 ± 2.3 ng/ℓ.

	Time (days)		
DDT in/on	4	11	20
Copepod lipid[a] (*ppm*)	1.56±0.05[b]	5.83±1.16	8.63±1.29
Total copepod[a] (*ppm*)	2.37±0.08	7.42±0.40	11.4±0.25
Sea water[c] (*pptr*)	30.5±0.5	34.3±0.6	43 ±3.0
No. copepods analyzed/container	83 ±4	67 ±3	51 ±1
% DDT in lipid	65.8±1.8	77.4±11.3	75.3 ±9.8

[a] Nanograms ^{14}C-*p,p′*-DDT per milligram dry weight *Calanus*.
[b] Mean ± SE of three containers.
[c] Nanograms ^{14}C-*p,p′* DDT per litre sea water.

TABLE 3. Uptake of ^{14}C-*p,p′*-DDT by adult female *Calanus finmarchicus* from sea water of 1337, 324 and 32 ng/ℓ.

		DDT concentration in/on copepod				
		Lipid				
DDT in sea water (*pptr*)	Time (*days*)	(*ppm*)[a]	(%)	Total (*ppm*)[a]	Sea water (*pptr*)[b]	No. copepods analyzed/ container
1337[b] ± 30	1	8.52±2.12[c]	38.3±4.2	21.66±3.16	1360±3	49±1
	3	40.52±2.11	80.2±1.1	50.43±2.39	1338±45	55±1
	7	57.72±7.71	56.3±9.3	104.31±7.17	1323±78	55±4
324 ± 13	3	7.46±1.29	67.8±6.4	10.99±1.42	296±12	39±2
	10	25.79±2.69	62.4±4.9	41.32±1.24	352±3	43±1
	16	36.92±8.31	51.5±11.1	71.67±1.16	395±11	32±3
32.5 ± 0.7	6	0.81±0.15	41.8±5.8	1.91±0.19	31±1	41±4
	20	1.23±0.16	27.7±3.9	4.46±0.13	34±1	37±3

[a] Nanograms ^{14}C-*p,p′*-DDT per milligram dry weight *Calanus*.
[b] Nanograms ^{14}C-*p,p′*-DDT per litre sea water.
[c] Mean ± SE of three containers.

TABLE 4. Values of ^{14}C-p,p'-DDT concentration in sea water, copepod surface-to-weight ratio, flux rates of ^{14}C-p,p'-DDT into copepods, and predictive equations using $k_j = 0.048$.

	W ± SE: *N* (*ng/ℓ*)	μ/α (× 10^{-6})	k_i (× 10^3/*day*)	*K* (× 10^{-2})	Fitted equations; *r*
A[a]	355 ±8; 15	8.94	2.0	38.3	$C = 136(1-e^{-0.048t})$;0.99
B	36.3±2.3; 8	8.94	2.7	52.3	$C = 19(1-e^{-0.048t})$;0.98
C	1337 ±30; 8	6.92	1.8	26.1	$C = 350(1-e^{-0.048t})$;0.97
D	324 ±13; 6	6.92	2.5	36.1	$C = 117(1-e^{-0.048t})$;0.98
E	32.5±0.7; 6	6.92	1.5	21.8	$C = 7.1(1-e^{-0.048t})$;0.96

[a] See Fig. 1.

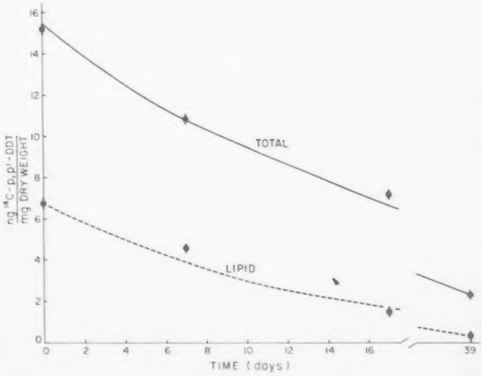

FIG. 2. Clearance of ^{14}C-p,p'-DDT from *C. finmarchicus* in flowing seawater tanks.

The results show close agreement to the model with k_i's ranging from 1.5 to 2.5 × 10^3 for adult females and from 2.0 to 2.7 × 10^3 for stage IV's and V's (Table 4). The difference in the surface to weight factor, μ/α, between adult and stage IV and V *Calanus* is small and could be deleted from the model with little change in the results. However, it is retained because Wildish and Zitko (1971) and Ware and Addison (1973) have indicated that the surface area of an organism is important in determining the uptake rate of organochlorines. If the model holds true for longer time intervals, the level of p,p'-DDT in copepods can be predicted at equilibrium, KW. This cannot be shown experimentally since copepods die due to the toxic effects of p,p'-DDT at high concentrations or succumb to starvation stresses at low concentrations.

Published values for ΣDDT in unfiltered sea water range from <1 pptr (Harvey et al. 1973) for the open ocean to 12.7 ppb (Seba and Corcoran 1969) for surface slicks in a contaminated marine bay. In this lab we have found that as high as 50% of the organochlorines in sea water are associated with particulate material removed by a 0.22 μm filter. If we use 1 pptr DDT in sea water for computations, C at equilibrium is equal to 312 ng DDT/g dry wt of *Calanus* or approximately 100 ppb wet wt. This level of contamination is well above reported values for oceanic zooplankton (Giam et al. 1973; Riseborough et al. 1972) but is approached in coastal waters (Cox 1971; Williams and Holden 1973; Woodwell et al. 1967). The inability of Harvey et al. (1973) to measure ΣDDT above the pptr level in oceanic water is therefore supported by the present work. It is impossible, however, to account for DDT present in natural plankton populations until the amount of DDT accumulated through feeding is known. Cox (1971) has shown that this can be quite considerable from several feeding experiments with euphausiids. Future work is required to evaluate this pathway in the marine copepods.

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Population Dynamics and Marine Mammal Management Policies[1]

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Some criteria for appraising population level relative to the maximal or carrying capacity point are listed. Simple population-dynamics models are then used to explore some of the criteria. Age at first reproduction does not seem as important as in some more prolific and shorter-lived species, being at most equivalent to a few percentage points of adult survival. Age-specific reproductive rates are very much the same for a number of pinniped species. For most marine mammal species, survival through immature stages is an unknown quantity, but appears to be a factor of major importance in determining population trend. Data on the Pribilof fur seals (*Callorhinus ursinus*) lead to the speculative conclusion that the maximum sustained yield (MSY) point may be to the right of the median value frequently assumed, so that "optimal" population levels may be closer to the asymptotic or carrying capacity level. Such a view is proposed as a conservative management policy.

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Nous présentons certains critères permettant d'évaluer le niveau d'une population en relation avec le point maximal ou de capacité de support. Nous nous servons ensuite de modèles simples de dynamique des populations pour explorer certains de ces critères. L'âge à la première reproduction ne semble pas être aussi important que chez certaines espèces plus prolifiques et de moindre longévité, étant tout au plus équivalent à un faible pourcentage de la survie au stade adulte. Les taux de reproduction à un âge donné sont à peu près les mêmes pour plusieurs espèces de pinnipèdes. Pour la plupart des espèces de mammifères marins, la survie des stades immatures est une quantité inconnue, mais semble être un facteur d'importance majeure dans la détermination de la tendance de la population. Les données sur les phoques de Pribilof (*Callorhinus ursinus*) nous mènent à la conclusion spéculative que le point de rendement maximal soutenu (RMS) est situé à droite de la valeur médiane fréquemment supposée, de sorte que le niveau « optimal » de population peut être plus rapproché du niveau asymptotique ou de capacité de support. C'est là le point de vue proposé comme politique conservatrice de gestion.

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PASSAGE of the United States Marine Mammal Protection Act of 1972 has resulted in changes of emphasis and philosophy in the management of marine mammals. Although the primary effects fall on species under U.S. influence or jurisdiction, the Act contains provisions that bear on international treaties and on U.S. trade with other nations. A central theme of the Act is the concept of an "optimum sustainable population" as a management goal. Clearly, the intent is to go beyond the notion of a maximum sustainable yield (MSY), towards a level that is "optimum" in a broader ecological context. This idea undoubtedly predates the Marine Mammal Protection Act by many years, but the Act appears to constitute the first effort to give it legislative status. Failure to achieve a precise definition of the concept is a natural consequence of the deficiencies in present-day knowledge about ecosystems.

We have been involved in an effort to identify criteria that may be useful in determining the status of marine mammal populations relative to the "optimum" concept. At present, good estimates of the actual numbers or densities are not available for most stocks of marine mammals. Consequently, we suspect that the best that can be done in the short term is to try to ascertain

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whether a given population may be in one of several broadly defined states. Two such widely used, although not necessarily accurately defined, states may serve as useful reference points. These are: 1) the population level variously known as "at carrying capacity," "maximal," or "asymptotic" (often denoted by K), and 2) the population level yielding the maximum sustainable yield (MSY). The MSY point is frequently determined by reference to the logistic growth curve, for which it falls at half the asymptotic level. One of the purposes of the present report is to point out that the MSY point is not known for most of the marine mammals, and to speculate that it may in fact be above the median level derived from the symmetrical logistic curve. There have been various suggestions that an "optimal" population level might lie between the MSY point and the carrying capacity, but this is a matter of conjecture, and subject to differences of opinion. It would thus be very helpful to know, at least roughly, the location of the MSY point before going on to consider the much more difficult problems associated with defining "optimal" conditions.

Criteria for Relative Population Level

In this section, we list a number of criteria that may be useful in establishing status of a population. Many of these criteria are best interpreted relative to maximal or carrying capacity population levels. For the most part, we do not know how to "scale" the individual items. Hence, the criteria constitute a set of benchmarks that may serve to indicate whether or not a population is in the immediate neighborhood of the carrying capacity level. If that level is known, and if the actual numerical abundance of the population can be measured, then the criteria suggested here will usually not be needed.

Twelve criteria are suggested in four groups as follows:

Behavioral Attributes

1) Antagonistic and/or displacement behavior.
2) Time spent in searching for food or in tending and feeding young.
3) Shifts in dietary components.

Individual Responses

4) Physical condition, including growth rates.
5) Incidence of disease and parasitism.

Reproductive Characteristics

6) Age at first reproduction.
7) Annual reproductive rates of mature females.

Populational Aspects

8) Age structure.
9) Survival rates, especially of young.
10) Occupancy of marginal range.
11) Rate of change of population size.
12) Effects on habitat or food base.

Many of the criteria are readily interpreted, assuming the observer has knowledge of these same traits in populations that are well below maximal levels. Those features associated with population dynamics may not be so readily interpreted and are discussed in the following sections. For example, in species having higher reproductive rates and lower adult survival than marine mammals, changing the age of first reproduction may have a dramatic effect on population growth rates. In marine mammals age of first reproduction does not seem nearly so important.

Models for Population Dynamics

Much of the contemporary theory of mammalian population dynamics utilizes some variant of the matrix models developed by Leslie (1945, 1948) for populations exhibiting age-specific reproductive and mortality rates. In practice, excluding humans and some captive or readily observed populations, we rarely have the detailed data required for such models. Instead, we usually have a fairly good notion of the age-specific reproductive pattern and some estimates of adult mortality rates. Survival through immature stages generally is not known, although there may be data on some portions of this period (e.g. from birth to weaning). Any analysis of population dynamics then depends on assumed or inferred values for immature survival.

Under the above conditions, a simpler model was used by Leslie (1966) and is followed here. We assume three survival rates: P_0 from birth (or embryonic stages) to 1 yr of age, P_1 from age 1 to age 2, and P for each year thereafter. Age-specific reproductive rates are available (usually as embryonic or ovulation rates) for many of the pinnipeds and appear to be quite constant from about age 6 or 7 onwards. For simplicity we denote this ("adult") rate as F and follow the usual convention of dealing only with females. An even sex ratio at birth is assumed, so that F is one half of the actual birth rate (i.e. $F = m_x$; cf. Leslie (1966): 292). For simplicity, P and F are assumed constant over all adult ages. Due to the longevity of marine mammals, this is an assumption that needs further evaluation.

As Cole (1954) has shown, the three "Lotka equations" for population dynamics can equally well be used in discrete form (given below) as in

TABLE 1. Age-specific reproductive rates and adult survival estimates for pinnipeds. Sample sizes in parentheses.

Age	Pribilof fur seal Chapman (1961, table 4)	Weddell seal Stirling (1971, table 4 and p. 145)	Harp seal Sergeant (1971)	Ringed seal Smith (1973)
4	0.05(171)	0.20(5)	0.02(79)	—
5	0.52(239)	0.50(16)	0.22(96)	0.41(22)
6	0.79(262)	0.75(12)	0.62(104)	0.59(42)
7	0.81(312)	0.90(10)	0.84(96)	0.85(63)
8	0.87(371)	0.86(14)	0.85(78)	0.86(98)
9	0.92(333)	0.94(18)	0.91(56)	0.91(114)
10	0.88(322)	0.72(18)	0.93(54)	0.94(117)
11	0.91(347)	0.84(12)	0.90(48)	0.97(77)
12	0.86(316)	0.90(10)	0.91(34)	
13	0.86(286)	0.92(12)	0.79(29)	
14	0.82(269)	0.50(6)	0.95(41)	
15	0.84(225)	0.50(4)	0.94(33)	
16	0.75(178)	0.80(5)	0.96(24)	
17	0.68(118)	1.00(1)	0.79(19)	
18	0.76(72)	1.00(1)	0.78(14)	
19	0.57(49)		0.86(14)	
20–26	0.51(39)		0.81(27)	
Adult survival estimates	0.89 Chapman (1973: 322) 0.86 Chapman (1961: 365)	0.83–0.85 Stirling (1971: 155), D. B. Siniff (unpublished data)	0.81–0.83 Ricker (1975b: 5,6) 0.87–0.88 Benjaminsen and Øritsland (1975)	0.85–0.88 Smith 1973: 35)

the continuous mathematics of the original derivation, providing reproduction occurs in a single, relatively short period each year. These equations depend on the assumption of a constant age structure, so that some caution is required in evaluating changing conditions; this is another feature that needs further study for marine mammals. For initial calculations, we assume reproduction commences at the adult rate (F) at some fixed age (age of first reproduction), giving the simplified forms on the right-hand side of the three basic equations:

$$1 = \sum_{x=a}^{x=\infty} \lambda^{-x}\ell_x m_x = \lambda^{-a}P_0P_1P^{a-2}F(1 - P\lambda^{-1})^{-1} \qquad (1)$$

where a = age of first reproduction, λ = finite rate of population increase ($\lambda = e^r$, where r = intrinsic rate of increase), m_x = age-specific reproductive rate, and ℓ_x is survival from birth to age x ($\ell_x = P_0P_1P^{x-2}$ for $x \geqslant 2$).

$$\frac{1}{\beta} = \sum_{x=0}^{\infty} \lambda^{-x}\ell_x = 1 + \lambda^{-1}P_0 + \lambda^{-2}P_0P_1 + \lambda^{-3}P_0P_1P(1 - P\lambda^{-1})^{-1} \qquad (2)$$

where β is sometimes called the "birth-rate per capita."

$$c_x = \beta\lambda^{-x}\ell_x = \beta\lambda^{-x}P_0P_1P^{x-2} \quad (x \geqslant 2 \text{ for right-hand side}) \qquad (3)$$

where c_x = proportion of population of age x.

The three equations above concern, respectively, 1) rate of increase of the population, 2) a birth-rate, and 3) age structure. The simplest interpretation is for data observed just after reproduction occurs, so that β is then c_0, the proportion of newborn in the population.

Reproductive Schedules and Survival Rates

Table 1 shows data for several species of pinnipeds and is particularly noteworthy for the closely comparable and quite constant reproductive rate at age 7 and beyond. The adult survival rates appear quite comparable, excepting the apparent lack of agreement as to the appropriate range for harp seals (*Pagophilus groenlandicus*). Only for the Pribilof Island fur seals are there extensive data on survival through the immature stages. These estimates are presented in Fig. 1 and were calculated from data given by Chapman (1973,

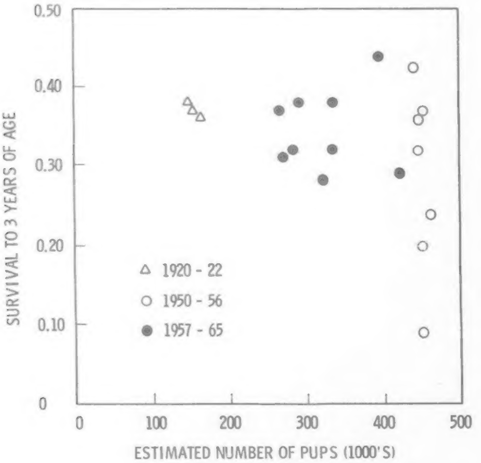

FIG. 1. Survival to age 3, St. Paul Island fur seals (*Callorhinus ursinus*). Data from Chapman (1973).

table 112) on pup population estimates and male recruitment at age 3, using Chapman's factor (1.1) for sex ratio of age 3 seals. These fur seal data strongly suggest a density-dependent immature mortality, with survival through age 3 being about 38% at the lower population levels. Smith (1973, table 15) assumes a higher rate for ringed seal (*Phoca hispida*) survival in the first 3 yr (0.44), while Stirling (1971, table 23) uses 46% survival to age 4 for Weddell seals (*Leptonychotes weddelli*).

If we assume 3rd yr survival in fur seals is equal to the adult rate (P), then the ratio of juvenile survival for the first 2 yr (P_0P_1) to adult survival for 2 yr can be estimated as:

$$\frac{P_0P_1P_2}{P^3} = \frac{P_0P_1}{P^2} = \frac{0.38}{(0.89)^3} = 0.54$$

where we use Chapman's (1973) value for adult survival. Using this estimate (rounded to 0.5) and setting $\lambda = 1$ (for equilibrium conditions), equation 1 can be rearranged to give:

$$F = \frac{1 - P}{0.5P^a} \qquad (4)$$

where a is age at first breeding. This relationship is shown graphically in Fig. 2, along with the approximate ranges of age at first breeding for some other marine mammal species.

The striking feature of Fig. 2 is the narrow range of adult survival corresponding to several years difference in age at first reproduction. Two tentative conclusions might then be deduced. One is that age of first reproduction may not be very important as a compensatory or regulatory mechanism in the circumstances considered here. It may nonetheless serve as an indicator of population conditions. Sergeant (1966) reported earlier maturity in harp seals as a likely consequence of heavy exploitation. However, Smith (1973) observed early maturity in a lightly exploited (Home Bay) ringed seal population in contrast to an apparently overexploited population (Cumberland Sound).

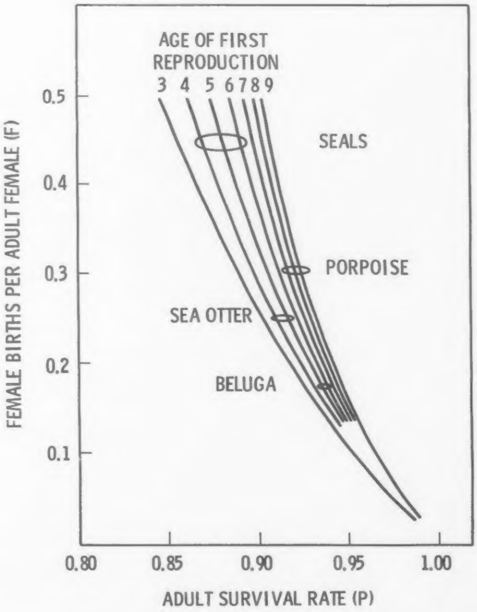

FIG. 2. Reproductive and adult survival rates for populations at equilibrium. Beluga (*Delphinapterus leucas*) data from Sergeant (1973), sea otters (*Enhydra lutris*) from Kenyon (1969), porpoise (*Stenella attenuata*) from Perrin et al. (1977), and seals from Table 1.

A second conclusion from Fig. 2 is that present population survey techniques for marine mammals are not likely to achieve the kind of precision required for measuring adult survival rates corresponding to the narrow range of such rates spanning the indicated ages of first reproduction. Two methods of estimating adult survival are in current use. Tag and recapture methods yield rather poor precision. From 4 yr of data, collected under good observational conditions, we (D. B. Siniff et al. unpublished data) obtained a standard error of estimate of nearly three survival percentage points for adult Weddell seals. The other method utilizes age structure data and with large samples apparently gives fairly good precision. Chapman and Robson (1960) gave an approximation to the variance of the estimated rate ($\hat{s}$) as:

$$\text{var}(\hat{s}) \doteq \frac{s(1-s)^2}{n}$$

where n is sample size. Thus, if survival is, say, 0.90, the standard error for a sample of 100 ages will be about one survival percentage point. Such estimates are, however, generally subject to unknown biases associated with the rather restrictive underlying assumptions of constant recruitment and constant population size.

Figure 2 gives an overall perspective of reproductive rates and adult survival rates for a number of marine mammal taxa, assuming populations are at equilibrium and that survival in the first 2 yr is a constant fraction (one half) of adult survival. These two assumptions may be examined by fixing the reproductive rate and plotting the survival rates for various rates of population increase (Fig. 3). For this purpose, we used the pinnipeds as being the best-studied group with the highest reproductive rate. We again assume a constant reproductive rate ($F = 0.45$), beginning at two ages of first breeding (4 and 6 yr). The ordinal scale of Fig. 3 is the ratio of survival for the first 2 yr to adult survival over the same time interval, i.e. we define $K = (P_0P_1)/P^2$ and rearrange equation (1) to give:

$$K = \frac{\lambda^{a-1}(\lambda - P)}{FP^a} \tag{5}$$

Figure 3 then depicts a plot of K against P for

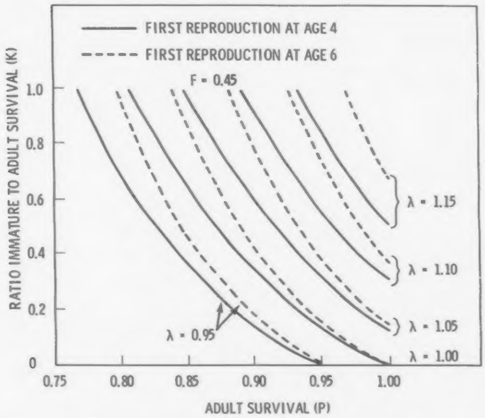

FIG. 3. Ratio immature to adult survival (K) plotted against adult survival (P) for various rates of population change (λ).

$a = 4$ and $a = 6$ at values of λ ranging from 0.95 to 1.15.

The actual pinniped reproductive data (Table 1) seem to be best approximated by the $a = 6$ line of Fig. 3. This can be illustrated by letting $\lambda = 1$ in equation (1) and rearranging the equation to calculate K for the actual age-specific reproductive rates:

$$\frac{1}{K} = \frac{P^2}{P_0P_1}$$

$$= P^a m_a + P^{a+1} m_{a+1} + \ldots + \frac{P^k m_k}{1 - P}$$

where k denotes the maximum age for which reproductive data are reported (Table 1) and this rate (m_k) is assumed to apply to all older seals. For a survival rate (P) of 0.85 this equation gives values of K of 0.84 (ringed seal), 0.91 (harp seal), 0.82 (Weddell seal), and 0.88 (fur seal) as compared to $K = 0.88$ for first reproduction at age 6 and $F = 0.45$ as calculated from equation (5) and plotted in Fig. 3.

The main conclusion to be drawn from Fig. 3 seems to be that population increases are not to be expected unless immature survival is quite high or adult survival is somewhat above the rates thus far reported for pinnipeds. This conclusion is relevant to the controversial and difficult question of the size of the western Atlantic harp seal population. While the population has recently shown evidence of a marked decline, the harvest records (Allen 1975) show that on the order of 200,000 pups were taken each year for over 20 yr (1949–71). Such a sustained harvest suggests that the population should have been nearly in equilibrium for at least a portion of the 20-yr interval. If adult survival was in the range of 0.85–0.90 (P), then from Fig. 3 we would expect K to be at least 0.40. Using equation (2) with $\lambda = 1$ (equilibrium) and rearranging, gives the proportion of pups in the population (β) as:

$$\frac{1}{\beta} = 1 + P_0 + P_0P_1 + \frac{P_0P_1P}{1 - P}$$

Figure 4 gives plots of β against P_0 (survival in the 1st yr of life) for $P = 0.85$ and $P = 0.90$ and various values of P_1. Given that $K \geqslant 0.40$, we have $P_0P_1 \geqslant 0.4P^2$. Since it is unlikely that P_1 (survival from age 1 to age 2) exceeds P, it seems quite probable that P_0 was not likely to be much < 0.40. Assuming some nonharvest mortality of pups necessarily existed, one can go on to reach the further conclusion that the total pup population was on the order of twice the harvest, and not more than one fifth the population. We

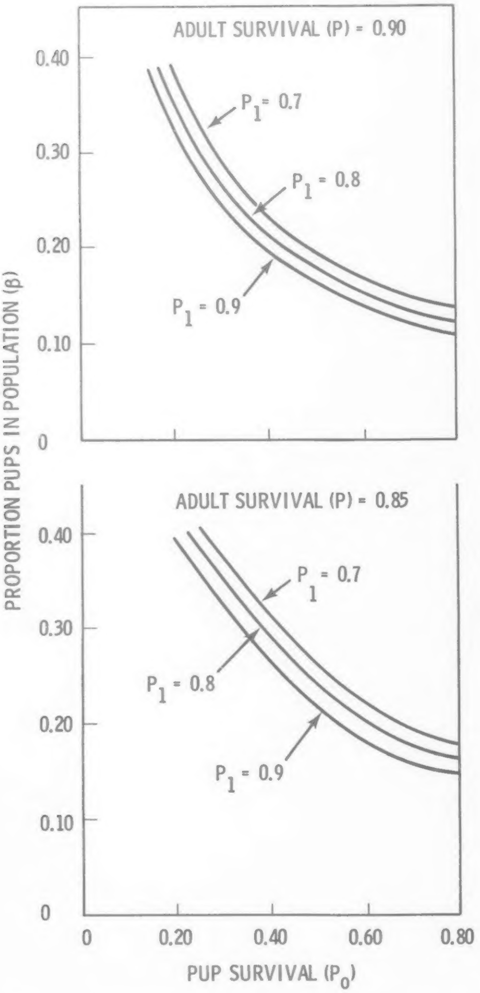

FIG. 4. Proportion of pups in the population for various survival rates.

are thus led to the not very surprising conclusion that pup production and total population were in the upper range of the published estimates.

Maximum Sustainable Yields and Optimum Sustainable Populations

The issue of maximum sustainable yield is basically one of density-dependent population regulation. Since little is known of such mechanisms in marine mammal populations, not much can presently be done beyond speculation. Figure 3 does suggest, however, that the upper limit is likely to be roughly 10% of the population for pinnipeds.

Chapman (1973) has attempted sustained-yield calculations for the Pribilof fur seal population. He used a "nonparametric" approach, which depends on interpolating for a calculated rate of change of recruitment (3-yr-old males) against population size (assumed proportional to pup population estimates). The data used by Chapman appear in Fig. 5, along with the slopes calculated in his table (apart from a misprint for the rate near the maximum population — given as 0.41 rather than 0.041). Chapman calculates the MSY point to fall at a slope of 0.076, giving an MSY value of 280,000 pups. Inspection of Fig. 5 suggests the speculative conclusion that the slope of the yield curve may be fairly constant until it reaches the immediate neighborhood of the maximum observed population. One might then suppose the MSY point to be substantially above Chapman's value. In the terminology of population biology concerned with "*r* and K" selection, it is apparent that marine mammals have the characteristics typical of "K-selected" forms, and thus the MSY point may be closer to the asymptotic population level (K) than previously supposed. Data presented by Gulland (1971: 459) on net reproduction in Antarctic

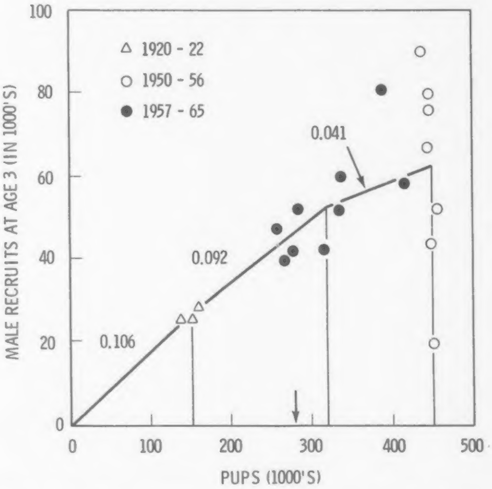

FIG. 5. Male recruits and pup numbers, St. Paul Island fur seals. Line segments and slopes indicate rate of change of recruitment against population size. Arrow indicates Chapman's (1973) estimate of MSY point.

blue whales also suggest a pattern like that of Fig. 5. However, Bonner (1975) gives some data on grey seals (*Halichoerus grypus*) that suggest early pup mortality to be linearly density dependent.

In fisheries management, the two most widely used yield curves stem, respectively, from the Ricker (1954, 1975a) and the Beverton and Holt (1957: 48–49) stock–recruitment curves. The first is skewed to the right (i.e. has a modal value to the left of the median and a long tail to the right), while the second is symmetrical (being closely related to the logistic curve). One essential difference is attributable to the high reproductive potential of certain fish species, which may be supposed to be "*r*-selected," i.e. capable of rapid population growth under favorable conditions. These species are typically characterized by the Ricker curves. We are here suggesting that marine mammals may well have a left-skewed yield curve, producing highest yields near maximal population levels.

Confirmation or refutation of our speculation about marine mammal yield curves will require much more data than are now available. Meanwhile, we propose that the assumption of such a model may constitute a conservative approach to management, assuming the absence of compelling reasons to maintain stocks at lower levels (e.g. conflicts with fisheries). The criteria proposed here may then serve as indicators of stocks approaching maximal levels, at which point harvests at percentage levels approaching those calculated as in Fig. 3 may be supposed to approximate maximum sustained yields. While the 12 criteria listed previously may serve to provide indications of a starting place for management (i.e. populations near maximal levels), we believe that efficient management may thereafter require accurate measurement of numerical abundance.

One of the disadvantages of the MSY concept is that it constitutes an abstraction, through which the harvested species is managed independently of the ecosystem of which it is a part. The idea of an optimum sustainable population as that level at which the given species contributes most to the well being of the ecosystem is thus attractive, and a goal to be desired and sought after. However, the difficulties in seeking that goal are obviously compounded by the need to consider the interactions and interrelations of many species simultaneously. Since we do not presently have the simpler answer (MSY) for most marine mammal populations, we can only propose that a conservative approach be attempted insofar as is practicable, given the many present and foreseeable future demands for human use of marine resources. Where feasible, such an approach should incorporate experimental management programs designed to test the response of stocks to different levels of exploitation.

Acknowledgments

Most of the items on the list of criteria resulted from discussions in connection with the work of the U.S. Marine Mammal Commission. D. G. Chapman and R. Eisenbud contributed extensively to these discussions. D. D. DeMaster provided several useful comments on an earlier draft of the paper.

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Host–Parasite Relationships Between Sockeye Salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae)

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KABATA, Z., AND B. COUSENS. 1977. Host–parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae). J. Fish. Res. Board Can. 34: 191–202.

The distribution of the parasitic copepod, *Salmincola californiensis*, on two size-groups (fry, fork length 3.2–5.8 cm; juveniles, fork length 10.2–27.0) of the sockeye salmon, *Oncorhynchus nerka*, is described. The existence of preferred sites for both groups is established. The distribution on the smaller fish differs from that on the larger in that on the former the copepods are most abundant in the region of pectoral and pelvic fins, whereas on the latter they are by far most common in the branchial cavity. Macroscopic and microscopic mechanical damage to the fish tissues, resulting from the presence and activity of the copepod, comprises injuries to gills, skin, muscle, and even bone. The "burrowing phenomenon" (failure on the part of the copepod to cease excavation of a completed cavity of implantation, resulting in perforation of body wall and penetration of the viscera) is reported upon for the first time.

KABATA, Z., AND B. COUSENS. 1977. Host–parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae). J. Fish. Res. Board Can. 34: 191–202.

Les auteurs décrivent la distribution du copépode parasite, *Salmincola californiensis*, sur deux groupes de longueur (alevins, longueur à la fourche 3.2–5.8 cm; juvéniles, longueur à la fourche 10.2–27.0 cm) de saumon nerka, *Oncorhynchus nerka*. Ils démontrent l'existence de sites préférés chez les deux groupes. La distribution du parasite sur les petits poissons diffère de celle des grands en ceci que chez les premiers, les copépodes sont plus abondants dans la région des nageoires pectorales et pelviennes, alors que chez les seconds, ils sont beaucoup plus abondants dans la cavité branchiale. Les dommages mécaniques macroscopiques et microscopiques causés aux tissus du poisson par la présence et l'activité du copépode comprennent des blessures aux branchies, à la peau, au muscle et même à l'os. Nous sommes les premiers à signaler le «phénomène de fouissement» (le copépode continue de creuser même après que la cavité d'implantation est terminée, ce qui entraîne la perforation de la paroi abdominale et pénétration dans les viscères).

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ANY parasite associated with the external surfaces of fish (including branchial and buccal cavities) can cause damage to these surfaces in one or more of the following ways: i) by the process of attachment, during which its prehensile or anchoring appendages or structures penetrate more or less deeply the integument of the fish, causing breaches in its protective cover; ii) by its feeding activities, also producing breaks in the integument and forming more or less extensive lesions; iii) when attached in a confined space, by exerting pressure on the adjacent tissues, resulting in their morbid modification; iv) indirectly, by inducing the fish to perform defensive movements which produce surface lesions on skin and fins (scraping against the bottom or submerged objects).

The nature and extent of the mechanical damage depends on the number and size of the parasites attached to the fish, as well as on the mode and place of their attachment and the mode of feeding. It is also related to the condition of the tissues which, in turn, is a function of the age and size of the fish.

The mechanical damage to the tissues belongs to the category of phenomena classed in an earlier publication (Kabata 1970) as "local effects." The

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importance of these effects to the overall health of the fish is directly proportional to the degree of their severity. They can be seen as no more than irritants. At the other end of the scale, they might constitute a serious impediment to the normal growth of the fish and even present a definite danger to its survival. A surface lesion is often seen as an "open gate" to secondary infections. While the danger of microbial infections resulting from such lesions is probably largely apocryphal, fungal infections which frequently follow external damage are real and dangerous enough.

This paper presents the results of observations made on the damage caused to the tissues of the sockeye salmon, *Oncorhynchus nerka* by the copepod *Salmincola californiensis* (Dana, 1852). Because the number and distribution of the copepods on the fish are reflected in the damage they cause, the description of the damage is preceded by an account of the distribution of the copepods on the fish. In view of the connection between the size of the fish and the effects of the ectoparasite upon it, two different size-groups of sockeye are examined and reported on.

Materials and Methods

Observations were made on two groups of fish. One, referred to below as "fry," consisted of 100 fish, size range 3.2–5.8 cm fork length. The other, referred to below as "juveniles," consisted of 120 fish, size range 10.2–27.0 cm fork length. (An additional 50 juveniles were used for count of injuries.) Fry were infected with *S. californiensis* by exposing them to contact with three heavily infected juveniles in a 109 × 51 × 43-cm tank. The exposure continued for 39 days at 10–12°C, during which the source of infection was augmented by addition of 11 batches of *S. californiensis* copepodids, each containing between 50 and 100 copepodids, hatched from eggs of mature females in a shaker bath at 10°C. In all, 11 such batches were added (on day 8, 10, 12, 14, 18, 19, 24, 26, 28, 30, and 37). Forty-eight fry died during that period. Each dead fish was carefully examined for the presence of various stages of *S. californiensis*. The remaining 52 fry were killed on day 39 and similarly examined. Observations on fish belonging to the juvenile group were made over a 1-yr period. The fish were kept as infection-holding stock in a laboratory tank at 9–12°C, where they were infected by exposure to *Salmincola*-carrying fish of the same size. The original infection came from sockeye smolts taken in Cultus Lake, B.C. Most records were made as the result of mortalities occurring naturally in the tank.

To describe the positions of attachment of copepods, the surface of the fish was divided into seven areas by three vertical lines and one horizontal (see Fig. 1 and 2). Vertical lines run from the anterior end of the dorsal fin to the anterior end of the pelvic fin, from the posterior end of the dorsal fin to the anterior end of the anal fin, and from the posterior end of the adipose fin to the posterior end of the anal fin. The area posterior to the third vertical line was undivided, whereas areas anterior to it were divided horizontally along the lateral line. The area anterior to the first vertical line and above the lateral line included also the top of the head. Black bands in Fig. 1 and 2 denote fin bases, whereas stippled bands parallel to the margin of opercula are defined as "branchial rims" and cover the outer margin of the branchial cavity directly under the operculum. They are divided into two more or less equal lengths. The gill filament surface area was estimated with the aid of a method adopted from Löpmann (1940). Individual gill arches were excised and placed on transparent plastic covering a sheet of graph paper. The outline of the gill was then pricked into the graph paper with the tip of a sharp needle and the number of squares within was counted and multiplied by two. The original outline of damaged gills was reconstructed by extrapolating a line linking the tips of intact filaments (see Fig. 8).

Tables 1 and 2 focus attention on grouping of *S. californiensis* on its host. Some records appear in them more than once. For example, a copepod attached on the pectoral fin appears both under "fins" and under "pectoral region." Hence, percentage figures exceed 100.

For histological examination, specimens were fixed in Formalin or Bouin's solution and embedded in paraffin. Several examples of each type of attachment site (e.g. gills, skin, etc.) were sectioned at 10 μm and stained with Mallory's one-step triple stain (Humasen 1967). Samples of corresponding uninfected tissues were treated in the same fashion. Examples subjectively selected as most representative are illustrated in Plates I–III.

Distribution of *S. californiensis* on Hosts

FRY

All 48 fry that did not survive the exposure period were infected. Of those killed on day 39, 8 remained free from the copepod. In all, 405 finds were recorded. They were divided into three categories: i) larvae, or all stages attached by frontal filaments ($n = 160$); ii) "adults," or all parasites attached by the bulla or old enough to have become detached from the frontal filament (i.e. adult and late chalimus IV females) ($n = 164$); and iii) frontal filaments to which no copepods were attached ($n = 81$). These filaments indicated positions of attachment of copepods which either did not survive beyond the initial stages of the life cycle or reached the adult stage and moved to another locality on becoming detached at the end of their larval life (for the life history of *S. californiensis* see Kabata and Cousens [1973]). The values for larvae and for unoccupied filaments may be combined to give

TABLE 1. Distribution of *Salmincola californiensis* on sockeye salmon, *Oncorhynchus nerka*, fry.

	Larvae (n = 160)		Filaments (n = 81)		Larval attachments[a] (n = 241)		Adults (n = 164)	
	No.	%	No.	%	No.	%	No.	%
Fins	31	19.4	59	72.8	90	37.3	8	4.9
Fin bases	58	36.2	18	22.2	76	31.5	107	65.2
Skin	56	33.7	4	4.9	60	24.9	25	15.2
Branchial region	15	9.4	—	—	15	6.2	24	14.6
Pectorial region (fins and fin bases)	52	32.5	23	28.4	75	31.1	68	41.5
Pelvic region (fins and fin bases)	19	11.9	25	30.9	44	18.3	19	11.6

[a] Larvae plus unoccupied filaments.

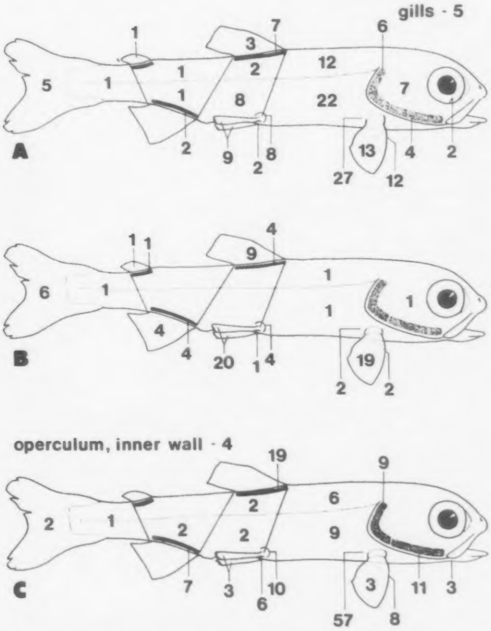


FIG. 1. Distribution of *Salmincola californiensis* on fry of sockeye salmon, *Oncorhynchus nerka* (in absolute numbers). A, Larvae; B, larval attachment filaments; C, adults.

a figure representing the total attachment of copepodids at various sites prior to postattachment mortality and/or maturation to the preadult stage (late chalimus IV) and migration (these two variables are not separable in this study).

The average number of copepods per fish was 3.2 (4.0 with filaments) and the largest number found on a single fish was 14 copepods and/or filaments. The distribution of the copepods on fry is shown in Fig. 1, and the most important features of this distribution are summarized in Table 1.

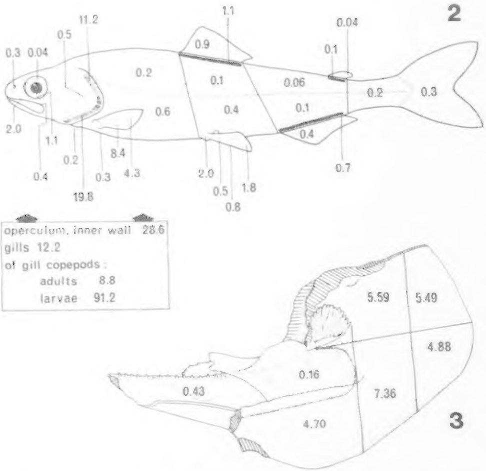
JUVENILES

In recording the presence of the copepod on the 120 fish in this group, no account was taken of the empty filaments, which were difficult to detect on fish in this size range. In all, 9361 adult and larval copepods were found, ranging from 20 to 390 per fish (78 average). The distribution of the copepods on fish, in percent of the total numbers, is shown in Fig. 2. Its salient features are summarized in Table 2.

The main difference between the distribution of the copepods on the fry and on the juveniles is in the preponderance of copepods within the branchial cavity of the latter. Almost three out of every four parasites occurred in that region. Few were attached to the gills (12.2%), almost all of those found there being immature. At the same time, attachment to the inner wall of the operculum greatly increased and accounted for more than 25% of the entire copepod population. The copepods were scattered over that surface with no apparent site of preference (Fig. 3), occurring also on the adjacent part of the mandible. A third of the population was densely crowded along the branchial rim, in some instances leaving no unoccupied space along the entire margin of the operculum. The bullae of the copepods were buried in the host tissues side by side, sometimes overlapping one another.

Damage to Host Tissues

As far as can be ascertained with the aid of macroscopic and microscopic examination, dam-



FIGURES 2 and 3. Distribution of *S. californiensis* on juvenile sockeye (in percentage of total number).

FIG. 2. Entire body; FIG. 3. Inner wall of operculum.

TABLE 2.. Distribution of adult *S. californiensis* on juvenile sockeye.

	No.	%
Fins	720	7.7
Fin bases	1301	13.9
Skin	196	2.1
Branchial region	6721	71.8
Pectoral region	1216	13.0
Pelvic region	477	5.1

age resulting from the presence and activity of *S. californiensis* on its salmonid host is mainly mechanical. There are indications that the parasite enters into metabolic exchanges with the fish through the mediation of the bulla, but at this stage we are still unable to determine whether the substances that may pass between the parasite and the fish are of pathogenic significance.

MACROSCOPIC OBSERVATIONS

The attachment of *S. californiensis* can be accomplished only at the cost of mechanical damage to host tissues. It is unlikely that the small punctures made in the integumental layers of the fish by the second antennae and the maxillae of the copepodid are of consequence in this respect. Much more extensive damage is caused by the implantation of the terminal plug of the larval frontal filament and, even more so, by the bulla of the young adult female. The damage is proportional to the size of the implantation cavity excavated by the parasite and further enlarged by the inflation of the bulla within that cavity (Kabata and Cousens 1973).

The feeding activity of *S. californiensis* results in damage inflicted by the mouth parts; the injuries are superficial and appear to have no serious consequences.

The presence of the copepod in the vicinity of soft tissues causes pressure to be exerted on them and may bring about partial or complete disappearance of those tissues. In some instances even hard tissues (bone) can be similarly affected.

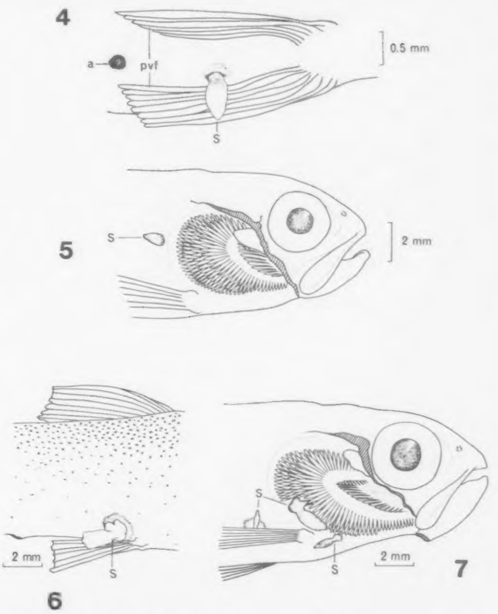
Since the type and the extent of damage inflicted depends on the site attacked and on the age and size of the fish, it will be described below separately for the fry and juveniles.

Fry — The injuries inflicted by *Salmincola* on 100 fry are shown tabulated and categorized below.

Type of injury

	No.
Superficial or deep lesions	
Axilla of pectoral fin	23
Region of pectoral fin other than axilla	7
Region of pelvic fin	19
Base of dorsal fin	9
Dorsum anterior to dorsal fin	9
Dorsum posterior to dorsal fin	4
Base of adipose fin	4
Caudal peduncle	3
Anal region	2
Ventral surface, anterior to pelvic fins	2
Ventral surface, posterior to pelvic fins	2
Gular region	1
Body flanks	1
Lesions penetrating body cavity	
Axilla or pectoral fin	7
Region of pelvic fins	5
Region of pectoral fins other than axilla	1
Abdomen	1
Lesions severing gular region	2
Lesions exposing skeleton	
Ventral side of caudal peduncle	1
Dorsum posterior to adipose fin	1
Lesion exposing heart	1
Erosion of opercular margin	5
Perforation of cranium exposing brain	1
Lesions involving partial or total loss of fins	
Dorsal fin	5
Pectoral fin	3
Pelvic fin	3
Adipose fin	3
Anal fin	2

The most spectacular injury caused by *S. californiensis* to salmon fry is the outcome of a hitherto unknown "burrowing phenomenon." We observed a number of copepods that continued to excavate the cavity of implantation long after they had reached a depth sufficient to accommodate the bulla. Several of them continued excavation into the visceral cavity of the fish and right through the viscera. The results are shown in Fig. 4–7.



FIGURES 4–7. Various body regions of sockeye fry, showing damage caused by *S. californiensis.*

FIG. 4. Abdominal wall; FIG. 5. Head with operculum removed; FIG. 6. Pelvic region; FIG. 7. Head with operculum removed. a — anus; pvf — pelvic fin; S — *Salmincola.*

Juveniles — Macroscopic observations of tissue damage observed on 170 fish of that group is tabulated below.

Type of injury	No.
Superficial or deep lesions	
Axilla of pectoral fins	3
Region of pelvic fins	2
Base of dorsal fin	2
Mandibular symphysis	2
Caudal peduncle	1
Subcutaneous hemorrhage	
Region of pectoral fin	3
Region of pelvic fin	1
Base of dorsal fin	1
Base of anal fin	1
Base of adipose fin	1
Body flanks	1
Damage to eyes	3
Damage to operculum	
Margins eroded	5
Perforations	5
Translucent bone patches	5

The fish of this group frequently display injuries caused by the presence, rather than the activity, of the parasite. As shown in Fig. 2, more than half of *S. californiensis* on juvenile sockeye are attached within the gill cavity (rims of the cavity and inner wall of the operculum). Their presence there, associated with the pressure exerted on them by the movements of the operculum and transmitted to the gill filaments, usually near their tips, results in the phenomenon of "crypting" (Friend 1941). This term denotes atrophy and eventual disappearance of the distal portions of gill filaments. Examples of gill arches afflicted with crypting are shown diagrammatically in Fig. 8. Examination of six juveniles for crypting produced results shown in Table 3.

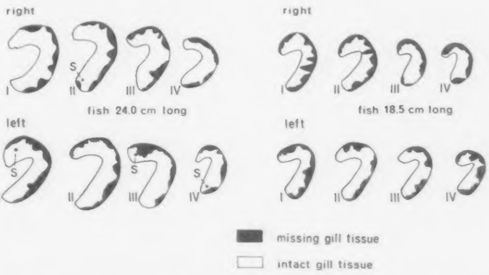


FIG. 8. Individual gill arches of two juvenile sockeye, showing crypting caused by *S. californiensis.* S — points of attachment of adult copepod.

In addition to the missing filament tips, the adjacent parts are affected by tissue reactions which render them nonfunctional for the purposes of respiration. The total loss of respiratory surface might, thus, amount to about one third of the surface originally available. (Besides parasites listed in Table 3, the latter three fish also carried on their gills 12, 15, and 29 larval copepods, respectively. Their presence, however, caused only slight damage to the tissues of the gills.)

Apart from injuries directly attributable to *S. californiensis* the fish (fry and juveniles) suffered as the result of rubbing against solid objects. This behavior was a response to the irritation caused by the parasite. Two fry had all their fins badly frayed; four pectoral, two pelvic and two anal fins were partially or completely lost. Partly or

TABLE 3. Damage caused by *S. californiensis* to gills of juvenile sockeye.

Fish length (*cm*)	Total gill surface (*mm*^{2})	No. adult copepods in gill cavity	% gill surface destroyed
21.5	2706	46	22
16.6	2034	24	16
24.0	2762	49	22
18.5	2374	39	25
22.5	3884	56	25
19.7	2146	37	23

completely missing fins were observed also among juveniles.

MICROSCOPIC OBSERVATIONS

The gills show the most vigorous response to the presence of *S. californiensis*. Sections through normal gill filaments are shown in Plate I, A and C; for comparison, sections through filaments affected by the presence of the copepod are shown in Plate I, B and D. Proliferation of the gill epithelium, sometimes accompanied by the hypertrophy of the epithelial cells, leads to partial or complete fusion of adjacent filaments. At less advanced stages of damage the filaments remain separate from one another, but thickening of their walls renders their affected areas nonfunctional as organs of gas and ion exchanges. Club-shaped sections of respiratory folds (Plate I, D) show that not only the epithelial cells respond to the irritation caused by the parasite; the palisade cells within the folds can rupture, causing formation of blood blisters. An extreme example of such blisters is shown in Plate I, E, which is large enough to be clearly visible to the naked eye. The blood cells which fill it can only be contained because of the greater than normal strength of the thickened walls of the respiratory fold. An unusual example of the bulla implanted between two gill filaments is shown in Plate I, F. Breaking through the branchial epithelium, the bulla enters into direct contact with the connective tissue of the filaments. In response to penetration of the bulla, a capsule is formed which encloses the anchor of the attachment organ and welds it securely to the gills. The final effect of this defensive reaction is diametrically opposite to the one that would benefit the fish. The attachment of larvae with the aid of the frontal filament produces a similar, though less pronounced, response from the branchial epithelium. In search of firm support, the copepodid places the terminal button of its frontal filament on the central rod of the gill filament (Plate II, A). The injured epithelial tissue then begins to obliterate the space between adjacent respiratory folds by filling it with the proliferating cells.

On the gills, and in other sites on the fish, the intrusion of the bulla below the epithelial surface evokes a comparatively mild reaction of the connective tissue. A delicate reticulum of fibers is found around the periphery of the bulla. Extravasation from ruptured small vessels is also fairly common around it (Plate II, D). Granulocyte invasion is often evident near the margins of the cavity of implantation; the concentration of these cells is particularly high opposite the subanchoral surface of the bulla (Plate II, C).

The effect of attachment of *S. californiensis* on the skin and muscle of the fish is illustrated in Plate II. E and F. The normal tissues (Plate II, E) comprise the following layers: epithelium (note abundant mucous cells); scales; dense connective tissue consisting of parallel, wavy fibers; loose connective tissue consisting of a meshwork of fibers; pigment; and muscle layer, showing individual muscle bundles. A section through a corresponding area with implanted bulla (Plate II, F) shows greatly increased thickness of the epithelial layer, now completely devoid of the mucous cells. The dense connective tissue layer has also become thicker. In contrast, the underlying loose connective tissue layer appears to have collapsed and become compacted, perhaps due to the loss of tissue fluids that took place during the activities associated with the implantation and inflation of the bulla. The myotomes directly in contact with the bulla also show signs of disintegration, either due to the pressure exerted upon them by the subanchoral surface of the bulla or to the influence of substances produced by the parasite and passed on to the tissues of the host. (Similar injuries are shown in Plate II, B, where the damage to the muscle fibers is particularly evident.) The reaction of the host tissues is only slight, not progressing beyond formation of a reticulum made up of connective tissue fibers. The injuries to the musculature are also restricted to the immediate vicinity of the bulla, and in particular its subanchoral surface.

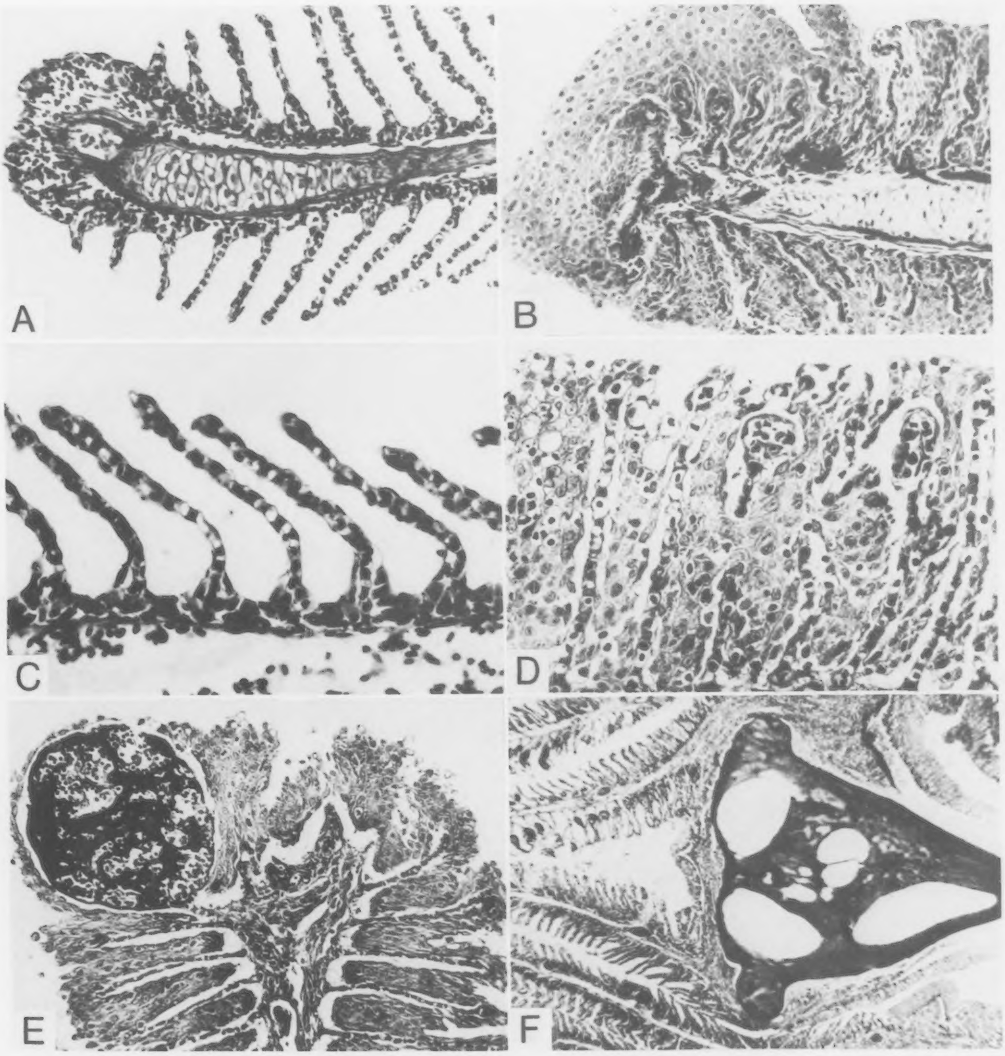


PLATE I. Sections through gills of sockeye salmon, *Oncorhynchus nerka*, uninfected and infected with *Salmincola californiensis*. A, Uninfected gill filament (× 125); B, infected gill filament (× 125); C, part of healthy gill filament (× 250); D, part of infected gill filament (× 250); E, blood blister on infected gill filament (× 125); F, bulla of adult copepod attached to two gill filaments (× 50).

The mode of feeding of *S. californiensis* can be classified as browsing. The surface of the host is scraped by the oral apparatus of the parasite, epithelial cells being ingested as the main item of its diet. Where the tissue of the host is thin (e.g. gills and inner walls of the operculum) and contains blood vessels near the surface, blood is also readily taken. Surface damage due to feeding activities is inflicted by the larvae, as well as by the adult parasites. Plate III, A, shows damage at the site of attachment of a larva (note the terminal button of the frontal filament). The cavity of implantation cuts down to the dense connective tissue layer. The scale layer has dis-

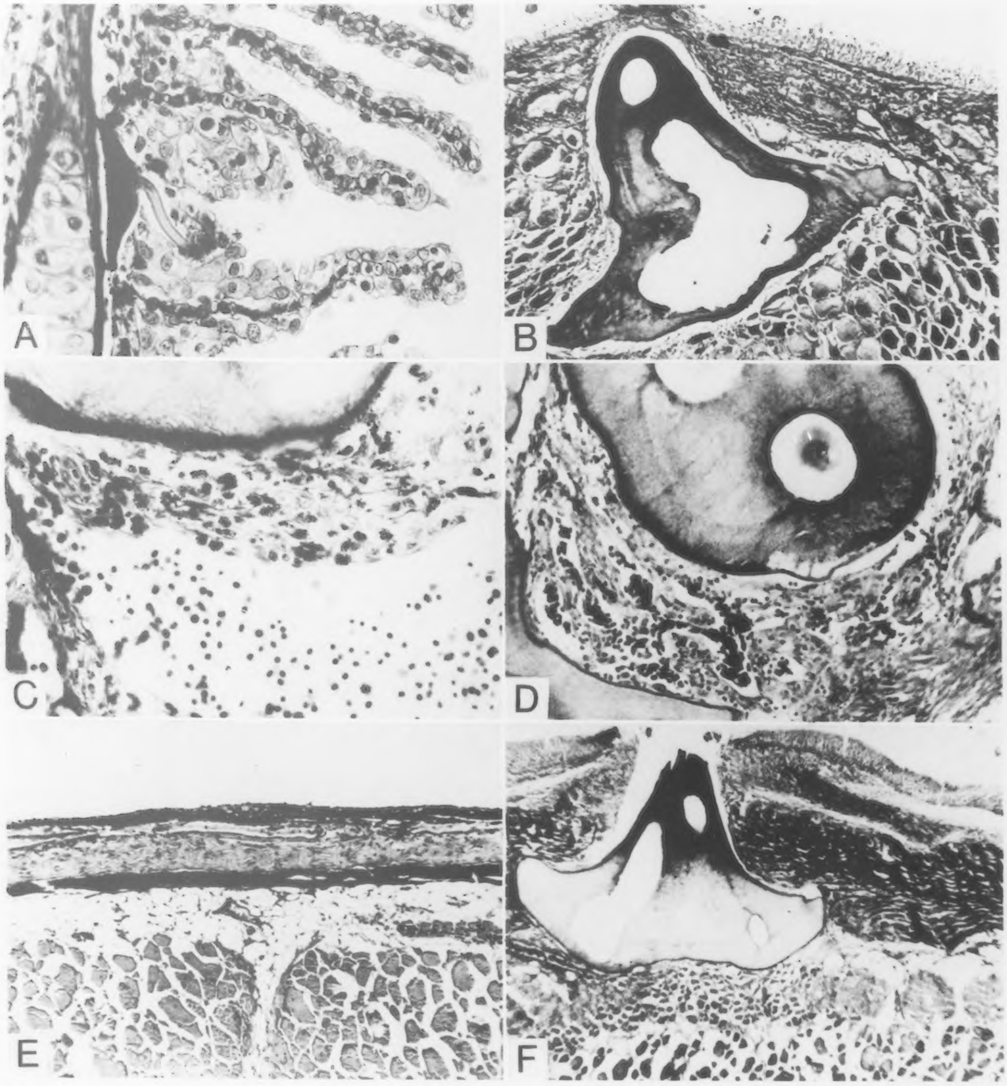


PLATE II. Sections through various tissues of sockeye, healthy and infected with *S. californiensis*. A, Gill filament with frontal filament of larval *S. californiensis* attached to supporting rod (× 250); B, bulla implanted in branchial rim area (× 50); C, granulocyte invasion of gill tissue adjacent to subanchoral surface of bulla (× 250); D, hemorrhagic lacunae around manubrium of bulla (× 125); E, normal skin and adjacent muscles (× 50); F, skin and muscle around implanted bulla (× 40).

appeared from the site, epithelial proliferation is rife, but no mucous cells are present. The area around the point of attachment is eroded due to feeding, which has aggravated the damage and resulted in the formation of a "feeding cavity." The connective tissue layers are, however, uninjured and the musculature is still protected from the intruder. Another type of damage associated with the parasites' feeding can be described as "shredding." It is shown in Plate III, B, taken from the flank of the fish, near the site of attachment of an adult female *S. californiensis*. Inflicted probably by multiple parallel strokes of scraping appendages, the injuries involve vigorous respon-

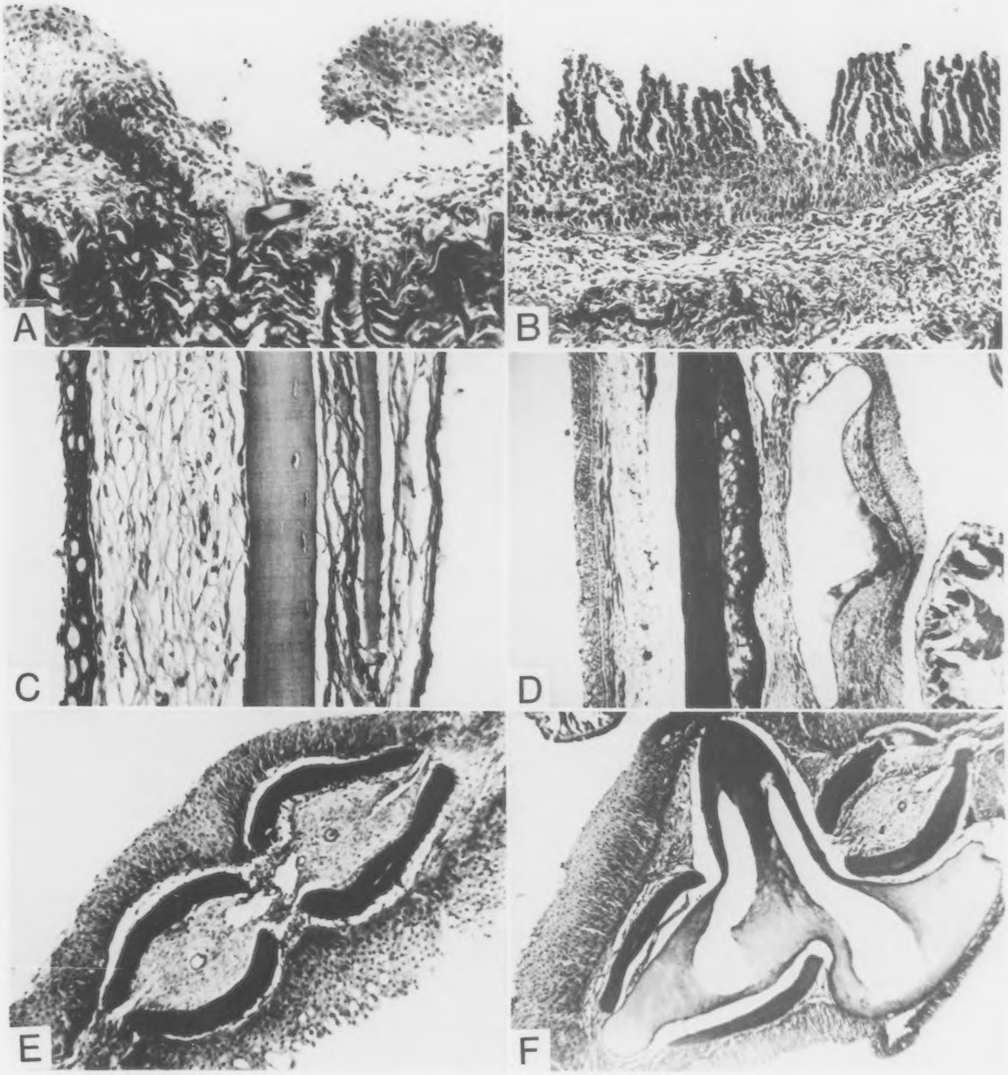


PLATE III. Sections through various tissues of sockeye, healthy and infected with *S. californiensis*. A, Frontal filament embedded in severely damaged skin of sockeye (× 125); B, epithelium of body wall damaged by feeding of copepods (× 125); C, normal operculum (× 125); D, operculum with implanted bulla (× 40); E, fin ray at some distance from implanted bulla (× 50); F, bulla implanted in fin ray (× 50).

sive proliferation of the epithelial layer and complete local disappearance of mucous cells and of the scale layer. Unlike the larval copepod, this parasite caused also local disruption in the dense connective tissue layer. The defense provided by the integument has almost been breached and the development of a macroscopic lesion was imminent.

The review of macroscopic injuries earlier in this paper showed that the harmful influence of the parasite can extend also to the hard skeletal tissues of the fish. An interesting example of bone damage is shown in Plate III. A section through the operculum, the inner wall of which is a favorite site of attachment of *S. californiensis*, is illustrated in Plate III, C. The opercular and subopercular bones appearing in the section consist of fine parallel lamellae, with a very thin periosteal layer of flat cells with darkly staining nuclei. In Plate III, D, the bulla of an adult female copepod has been implanted in close proximity to the bone. Although there is no direct contact between the bulla and the bone, pathological changes in the latter are quite evident. The bone directly under the subanchoral surface of the bulla and for some distance around it has undergone profound changes. Irritation of the periosteal cell layer during bulla implantation has resulted in osteogenic activity and the buildup of a layer of spongy bone. This proliferation has produced a local thickening of the opercular bone in the immediate vicinity of the bulla, to a maximum of almost twice its original thickness (as indicated by the densely staining lamellar layer farthest from the bulla in the figure). The tissues covering the bone, and in particular the epithelium, show a substantial increase in thickness. Areas subject to excessive or prolonged damage during implantation, or to continuous pressure and abrasion by the body of the adult female copepod, sometimes become denuded of these covering tissues, exposing the bone. The regenerative and proliferative capacity of the periosteal cells is quickly lost under these circumstances, to be followed by degeneration. The bone may become translucent and seemingly crystalline, losing its lamellar structure. Eventually the solidity of the bone is destroyed. Further progress of this process occasionally results in perforation of the operculum.

Plate III, E, shows a section through a normal fin ray, consisting of two pairs of bone lamellae enclosing connective tissue and a pair of blood vessels. The copepod excavated a cavity of implantation which cut between and penetrated below two adjacent lamellae (Plate III, F). When first extruded, the bulla is thin and can be easily inserted into a narrow space (Kabata and Cousens 1973). As the copepod subsequently inflated the bulla within the cavity, it increased in size and caused serious damage to the fin ray. Although the bones were not broken, the circulation within the ray was disrupted. The consequence of this injury can be more serious than the injury itself, since necrosis of the tissues distal to the point of attachment might be precipitated and result in destruction of a substantial part of the fin.

Discussion

DISTRIBUTION

The results show that the major sites of larval attachment on fry are the fins, fin bases, and skin, representing 37.3%, 31.5%, and 24.9% of the total larval attachments, respectively. In view of the much smaller total surface area of the fins, and especially the fin bases, in comparison with that of the skin, these figures suggest that the fin regions may be preferred by some method of site selection by the attaching copepodid. Only one in four copepodids becomes attached to the scale-covered skin, although this surface offers the largest attachment area, and over 60% of these are found on the flanks of the fry anterior to the dorsal fin, usually close to the margins of the opercula. A disproportionately large number settles on or around the pectoral fins, again an area very small in comparison with the total body surface. The combined regions of both pectoral and pelvic fins account for almost half (actually 49%) of the total attachments and 44% of the larvae.

There is some evidence suggesting that the chief stimuli initiating swimming in the infective copepodid larva, and hence, host-seeking and attachment, are a shadow response and a response to any disturbance in the surrounding water (B. Cousens unpublished data). Thus, forward movement and particularly movement of the pectoral fins and opercula, as well as the respiratory current through the branchial chamber would likely tend to cause the most frequent copepodid-host contact on or in the general vicinity of the pectoral fins and opercula, and a decreasing frequency of contact and attachment with distance away from this region. The results of this study appear to support this hypothesis (Fig. 1 and 2).

The delicate tissues of the fins, although sufficiently firm to support the frontal filament of the larval copepod, do not seem to be an entirely suitable substrate on which to become permanently established. The fins accounted for 37.3% of the total larval attachments, but supported only 19.4% of the attached larval population, the difference between these figures being due to the large proportion of unoccupied filaments (73%) found on the fins (Fig. 1B). This high proportion of abandoned larval attachment sites may imply a higher larval mortality on the fins than elsewhere. This cannot be stated with certainty from the present data, as mortality was not distinguished from filament abondonment and migration to a new site at maturity, but the alternate hypotheses (i.e. differential changes in rates of larval settlement and/or survival with site and time) seem far less likely. On the other hand, the fin bases accounted for 31.5% of the total larval attachments and supported a similar proportion (36.2%) of the attached larvae, suggesting that perhaps larval mortality is somewhat lower in these areas. The branchial region accounted for only about one tenth (9.4%) of the larval population, but no abandoned filaments were observed. This is likely due in part to the difficulty of detecting unoccupied filaments in this region, but their apparent scarcity in the presence of attached larvae and adults may also imply rather good larval survival on and about the gills, particularly in view of the high densities of larvae of all stages found in the branchial region of larger hosts (Fig. 2).

Adult copepods (Fig. 1C) were relatively scarce in the branchial region of fry, representing 14.6% of the adult population. Of these, none were found on the gills themselves, and few on the inner wall of the operculum; most were located along the branchial rim. Very few adults occurred on the fins, but the bases of the fins accommodated about two thirds (65.2%) of the adult copepod population. The axillae of the pectoral fins alone harbored more than one third of that population.

The fin bases of fry showed a marked increase in the percentage of the adult copepod population supported over the percentage of the larval population and total larval attachment. Concurrently, skin and fins showed considerable decreases in the proportion of adult copepods from those of larvae and total larval attachments. The phenomenon was particularly obvious in the regions of the paired fins, but the unpaired fins all show the same effect to a lesser degree (Fig. 1). This evidence strongly suggests that the abundance of adult *S. californiensis* at fin bases and their apparent preference for this site in fry is the result of preadult chalimus IV females abondoning the sites of heavy larval settlement on fins and scale-covered skin and moving to the more firm and, at the same time, penetrable tissues of the fin axillae and bases for bulla implantation. Firmness and penetrability are not essential qualities of the substrate for larval attachment as such deep penetration is not required. The terminal button of the temporary larval attachment filament is inserted just below the epithelium and is usually cemented to some shallow but solid structure such as a gill or fin ray, the surface of a scale, or even fibrous connective tissue. However, these qualities are essential for secure and permanent anchoring of the much larger adult female parasites.

In the branchial cavity of juveniles the main site of larval attachment is the gills, while the majority of preadult females migrate from the delicate tissues of the gills to the firmer tissues of the adjacent operculum of the branchial rim for permanent establishment as adults.

Adult sockeye infected with *S. californiensis* in nature frequently carry large numbers of adult copepods on the gill filaments. Thus, with an increase in size, the filaments provide a much more suitable substrate to the adult copepod, eventually becoming one of the major sites of attachment in mature fish.

A marked drop in the proportion of attached copepods occurs in the region of the paired fins. Whereas on fry 49% of all the copepods, adult and larvae, were attached there, on the juveniles the corresponding figure was $< 20\%$. The pectoral axilla still appeared to be the site most favored outside the branchial chamber and harbored 8.4% of the copepod population. *Salmincola californiensis* can become attached and is capable of maturing on such sites as the maxillary fold, the rim of the orbit, on the eyeball, in the nose, and inside the buccal cavity (2%). When the surface area of that cavity is compared with the expanse of the exposed skin which supports 2.1% of the copepod population, the conclusion that not all the attachment sites are equally acceptable to the copepod is inescapable; all the sites of adult attachment, with the exception of the skin, have in common the absence of a scale layer. In these and larger fish the scale layer can be expected to be considerably less penetrable to preadult *Salmincola* than that of the fry discussed previously, and hence would present an even greater barrier to adult attachment as the size of the host increases.

Considering the pattern of distribution on fish of different sizes and ages, one arrives at the following conclusions: a) *S. californiensis* does select its site; b) when the most preferred sites are filled, it can become attached and survive on less preferable ones; c) the site should preferably be devoid of scales and be firm enough to give anchorage to the frontal filament or the bulla: d) the attachment conditions required by the larval stages differ from those most suitable for the adults, the main difference between them being the depth and degree of firmness of the host's tissue sufficient to support implantation, hence, frequent change between the position of attachment of the larva and the adult.

"BURROWING PHENOMENON"

We suggest the following tentative explanation of this curious activity of the preadult female copepod (chalimus IV). Once engaged in the process of excavation, following a laborious search for a suitable attachment site, the female is driven by a "burrowing reflex." The action of the reflex continues until the cavity is large enough to receive the bulla and until it has reached tissues firm enough to assure completely safe implantation. (Only very rarely does one find a copepod fixed in soft tissues.) When these two conditions are satisfied, the reflex is deactivated and the female proceeds to implant the bulla. Should the cavity fail to reach suitable supporting tissue, the burrowing continues. The cavity becomes deeper, its periphery might erode (Fig. 6 and 7) and the parasite enters more and more deeply into the body of the host (Fig. 4 and 5). In fry, which have particularly soft tissues, each burrowing female is potentially lethal. Some fish, however, can survive burrowing, as suggested by the report of Adams (1956) on *S. falculata* (=*S. californiensis*) attached to the pericardium of a sockeye salmon 8.4 cm long.

TISSUE DAMAGE

The general picture of mechanical injuries inflicted on the fish by *S. californiensis* shows that the epithelium is the tissue most seriously affected. Not only is it most extensively damaged, but its reaction is also more vigorous than that of other tissues. The scope of this response is such as to become sometimes deleterious to the fish (e.g. in gill injuries). The epithelial reaction to mechanical injuries caused by this copepod is quite typical; it resembles those described previously for other parasitic crustaceans. A review of these injuries was published by Kabata (1970). A brief reference to the epithelial response provoked by this species of copepod was published by Hoshina and Suenaga (1954). No other account of mechanical damage caused by *Salmincola* is known to us.

The vigorous response of the epithelium is not matched by that of the connective tissue. The dense connective tissue layer around the rim of the implantation cavity increases in thickness, but a capsule rarely forms around the bulla. The development of the loose and very sparse reticulum of connective tissue fibers and some infiltration of the surrounding tissues by various blood cells are the only signs of incipient inflammation. There is no intimate fusion of the bulla and the host tissue which is common in marine Lernaeopodidae (cf. Kabata and Cousens 1972). The injuries sustained by the muscle tissues directly below the anchor of the bulla could be caused by metabolites excreted by the parasite through that organ. On the other hand, the pressure exerted by the bulla could also be instrumental in causing this particular damage, combined with possible osmotic disruption during the attachment process.

An interesting aspect of mechanical damage caused by *Salmincola* is the bone injury, though the mechanism of such injury is not fully understood. Severe but short-lived irritations of the bone during attachment and bulla implantation often result in local proliferation of spongy bone, while more prolonged forms of irritation or pressure may result in serious degeneration or complete destruction of the bone in the irritated area, perhaps due to exhaustion of the reparative capacity of the osteogenic cells. The fact that such injury appears possible in some cases, even in the absence of apparent direct contact between the bone and the parasite, suggests that an intermediary agent may be involved, or that injurious local chemical changes may be induced in the tissues of the host by the parasite. The possibility of an injurious metabolite, of either host or parasitic origin, is raised again, although there is no direct evidence of such a substance at present. More than one mechanism may be operative, or the mechanisms may differ in type or sensitivity with location on the host.

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Immunization of Pacific Salmon: Comparison of Intraperitoneal Injection and Hyperosmotic Infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* Bacterins[1]

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Two methods of immunizing fish, intraperitoneal (i.p.) injection and hyperosmotic infiltration, were compared for control of vibriosis and furunculosis in pen-reared coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*). Both methods provided significant protection against vibriosis under field test conditions. In coho salmon, hyperosmotic infiltration provided the best protection and fastest rise in antibody titer of seven treatments tested. In chinook salmon, hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* vaccines resulted in 83.3% survival in comparison with 28.7% survival in controls. Both i.p. injection and hyperosmotic infiltration of *V. anguillarum* and *A. salmonicida* bacterins resulted in production of serum antibodies specific for each respective pathogen. Vaccination with bivalent *V. anguillarum–A. salmonicida* vaccines produced antibodies to both pathogens, and provided protection against vibriosis. Growth rates of vaccinated coho salmon were not significantly different from controls.

ANTIPA, R., AND D. F. AMEND. 1977. Immunization of Pacific salmon: comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins. J. Fish. Res. Board Can. 34: 203–208.

Les auteurs comparent deux méthodes d'immunisation de poissons, injection intrapéritonéale (i.p.) et infiltration hyperosmotique dans le contrôle de la furonculose chez des saumons coho (*Oncorhynchus kisutch*) et chinook (*O. tshawytscha*) élévés en enclos. Les deux méthodes assurent une bonne protection contre la vibriose dans les conditions d'essai sur le terrain. Chez les saumons coho, l'infiltration hyperosmotique donne la meilleure protection et l'élévation la plus rapide du titre d'anticorps de sept traitements essayés. Chez les saumons chinook, l'infiltration hyperosmotique de vaccins de *Vibrio anguillarum* et d'*Aeromonas salmonicida* résulte en une survie de 83.3% comparativement à 28.7% chez les témoins. L'injection i.p. et l'infiltration hyperosmotique de bactérines de *V. anguillarum* et de *A. salmonicida* causent toutes deux la production d'anticorps sériques spécifiques à chacun des organismes pathogènes respectifs. La vaccination avec vaccins bivalents de *V. anguillarum* et de *A. salmonicida* produit des anticorps contre les deux agents pathogènes et protège contre la vibriose. Le rythme de croissance des saumons coho vaccinés ne diffère pas significativement de celui des témoins.

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THE ability of teleost fish to develop active immunity to bacterial pathogens has been well established in laboratory experiments involving oral and parenteral vaccination (Duff 1942; Krantz et al. 1963; Ross and Klontz 1965; Post 1966; Fujihara and Nakatani 1971; Anderson and Nelson 1974). Transition from successful laboratory methods to larger scale field tests has been accompanied by inconclusive results (Snieszko 1970); the number of effective field trials has been small (Overholser 1968; Fryer et al. 1972). Oral immunization has been the most tested system, although intraperitoneal (i.p.) injection has

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been shown to be applicable to large-scale immunization (Antipa 1976; A. Novotny, National Marine Fisheries Service, personal communication). However, both of these methods of immunization have many inherent disadvantages and there is a great need for a practical vaccine delivery system that exhibits high efficacy, low cost, minimum stress on fish, fast processing time, and is not limited by fish size.

The purpose of this investigation was to compare the efficacy of hyperosmotic infiltration (HI) (Amend 1976) with i.p. injection in controlling vibriosis (*Vibrio anguillarum*) and furunculosis (*Aeromonas salmonicida*) in pen-reared coho salmon (*Oncorhynchus kisutch*) and chinook salmon (*O. tshawytscha*) in marine aquaculture conditions.

Materials and Methods

VACCINE PREPARATION

The methods and isolate used in preparation of *V. anguillarum* bacterin were identical to those used by Antipa (1976), except that glucose was added to the culture 19 h after inoculation (final concentration 0.5%) and the bacteria were killed by adding Formalin (final concentration 0.3%) for 24 h at room temperature.

We grew *A. salmonicida* (isolate C74-11; from T. P. T. Evelyn, Biological Station, Nanaimo, B.C.) in 3% Trypticase® Soy Broth (Baltimore Biological Laboratories) containing 0.028% Antifoam A Spray (Dow Corning). Bacteria were grown for 24 h at 27°C in a Virtis fermenter at 200 rpm with 4,000 cm^3/min aeration. The pH was maintained at 6.8–7.3 with 1 N NaOH. Glucose was added after 18-h incubation (final concentration, 1.0%). Cells were killed by adding Formalin (final concentration 0.3%) and agitating the solution (200 rpm) for 24 h at room temperature.

The bacteria for use with i.p. injection were harvested by continuous centrifugation of the cells at 23,500 g (4°C). Cells were then washed twice with sterile 0.85% saline and finally resuspended in 0.85% sterile saline at a concentration, in dry weight of bacterial cells, of 6.7 mg/ml (wet weight of bacterial cells 23.9 mg/ml). Bacterins for i.p. injection were stored at −20°C until use and were tested for sterility and safety in the laboratory before field use. All injections referred to in the text or figures were i.p.

Bacterins for HI were not centrifuged and were standardized by the Lowry method of determining protein content (Lowry et al. 1951), using bovine serum albumin as the standard. All *V. anguillarum* harvests were pooled and standardized at 0.48 mg protein/ml (2×10^9 viable bacteria/ml) by addition of sterile 0.85% saline. The *A. salmonicida* bacterin was standardized at 0.52 mg protein/ml (2×10^{10} viable bacteria/ml). Oxytetracycline was added to the HI bacterins at the rate of 60 μg/ml of bacterin as a preservative. Hyperosmotic infiltrated bacterins were tested for sterility, safety, and potency and were stored at 4°C before use.

SOURCES OF FISH

The coho salmon fry used (14,000; 12 g mean wt) were obtained from Trout Lodge, Inc., Ephrata, Wash.; chinook salmon fry (1,000; 5 g mean wt) were supplied by the Big Beef Creek Fish Research Station of the University of Washington.

VACCINATION PROCEDURES

We conducted the injections with automatic pipetting syringes (1 ml Cornwall) as described by Antipa (1976). The inoculum volume of 0.15 ml/coho salmon contained 1.01 mg bacteria dry wt (3.59 mg wet wt).

The HI method consisted of placing the fish in a vacuum chamber containing equal quantities of bacterin and 20% sterile urea. A spherical plastic desiccator (25 cm inside diam, Dura-Vac brand, Ace Glass, Inc.), used as the vacuum chamber, was connected to an electric vacuum pump. The pressure was reduced from ambient (760 mm Hg) to 380 mm Hg, held for 10 s, and then returned to ambient for 30 s; the entire cycle was repeated twice. Fish that had not been fed for 24 h were placed in the vacuum chamber at the rate of 450 g/ℓ of vaccine–urea solution. The fish were placed in aerated holding tanks after HI. Each liter of vaccine solution was reused for 10 vaccinations before being discarded. Temperature of the vaccine solution was kept within ±5°C of the ambient water temperature (11°C). A second HI (booster) was given 13 days after the first, by identical methods.

The coho salmon were immunized in the freshwater hatchery and held 5 wk postinoculation in fresh water (mean temperature 14°C) before transfer to the saltwater rearing site. Silver Cup salmon feed (formula No. 2, Murray Elevators, Murray, Utah) containing standard levels of oxytetracycline (8.8 g Terramycin®/100 kg fish per day) was fed to all experimental groups of coho salmon during their postvaccination freshwater residence.

Chinook salmon were vaccinated and finclipped in fresh water and held for 4 wk after vaccination before they were transferred to salt water (finclipping wounds healed prior to saltwater entry). Oregon Moist Pellets (formula No. 2, Moore-Clark Company, LaConner, Washington) without drug supplements were fed to the chinook salmon throughout the experiment.

DESIGN FOR SALTWATER EXPERIMENT

The saltwater aquaculture site was located at the Weyerhaeuser Company log storage area in Henderson Inlet, southern Puget Sound. Fifteen floating nylon mesh net pens, 2.5 × 2.5 × 2.2 m, were used in the study. Seven groups of coho salmon were given the following treatments: 1) HI *V. anguillarum* bacterin, 2) injected *V. anguillarum* bacterin, 3) injected mixture of 50% *A. salmonicida* and 50% *V. anguillarum* bacterin, 4) injected *A. salmonicida* bacterin, 5) injected saline, 6) HI well water, and 7) untreated control. Each treatment was replicated in two saltwater pens containing initial populations of 1,000 fish each.

Two groups of chinook salmon, one HI with *V. anguillarum–A. salmonicida* bacterin (473 fish) and one (the control) HI well water (495 fish), were held in a single pen. The adipose fin was removed from the chinook HI with well water, to distinguish them from the vaccinated fish.

All coho were fed unmedicated Silver Cup salmon feed at the rate of 2.24% body wt/day while in saltwater pens. All chinook were fed unmedicated Oregon Moist Pellets at 2.40% body wt/day throughout the experiment.

DIAGNOSTIC AND SEROLOGICAL TESTS

Diagnostic methods were the same as those of Antipa (1976) except the slide agglutination test (Fish Health Section 1975) was added for identification of *A. salmonicida.*

Serum agglutinins to *V. anguillarum* and *A. salmonicida* were determined by standard microtiter procedures. The caudal penduncle was severed and blood was collected in microhematocrit tubes. Formalin-killed *V. anguillarum* (HI-163) and *A. salmonicida* (C74-11) at concentrations of 5 mg wet wt/ml were used as the antigens in the agglutination tests (0.3% phenol was added as a preservative). After 18 h of incubation at 7°C, the endpoint titer was determined with a 15× dissecting microscope.

Results

Both hyperosmotic infiltration and injection of *V. anguillarum* bacterins in coho salmon provided protection against vibriosis in comparison with control groups (Fig. 1). All postvaccination phases of this experiment (holding in fresh water, transfer to salt water, and saltwater rearing) were accompanied by mortality of test fish. Mortality was highest during the saltwater rearing phase. In the seven treatment groups of coho salmon total mortality was significantly lower in fish hyperosmotic infiltrated with *V. anguillarum* bacterin than in the other groups (chi-square test, $P < 0.001$).

Postvaccination freshwater mortality was highest (17.8% mortality) in the group injected with *V. anguillarum* and *A. salmonicida* bacterin, and second highest in the group injected with *A. salmonicida* bacterin (3.8% mortality). A gram-negative enterobacterium was isolated from 86% of the 49 moribund fish examined (kidney disease–*Corynebacterium* sp. and furunculosis were also detected in a small percentage of the fish). The enterobacteria had biochemical reactions similar to those of the enteric redmouth disease (ERM) isolates studied by Ross et al. (1966), but did not agglutinate with ERM antiserum. Sensitivity tests indicated that the ERM-like isolates were tetracycline sensitive in vitro. Oral Terramycin® chemotherapy at standard levels (8.8 g/100 kg fish per day) was initiated 1 day postvaccination in all groups of coho salmon and was continued throughout the freshwater holding phase. It is not known what value chemotherapy provided because all groups were treated, but it is apparent that the losses were more severe in the injected than in the HI groups.

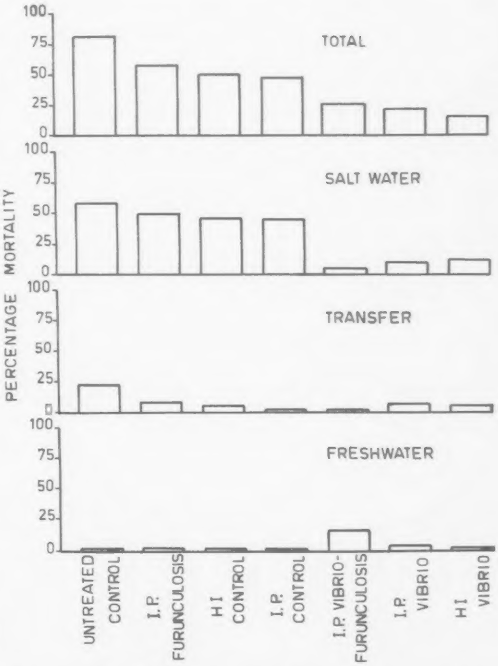

FIG. 1. Coho salmon mortality components for seven experimental groups (i.p. = intraperitoneal injection, HI = hyperosmotic infiltration). Freshwater mortality consists of postvaccination mortality; transfer mortality consists of mortality during shipment to saltwater site and during the first 24 h in salt water; saltwater mortality extends over 4 mo of pen rearing.

As transfer mortality (Fig. 1) included that sustained during truck transport to the saltwater rearing site (about 400 km) and during the first 24 h in saltwater pens, the stress of saltwater adaptation contributed to these losses. Transfer losses ranged from 22.9% (untreated controls) to 1.6% (fish-injected with *V. anguillarum–A. salmonicida* bacterin). No fish pathogens were detected in 28 of the 30 moribund fish sampled (kidney disease was found in the other 2).

Vibriosis epizootics were the most important cause of saltwater mortality in this experiment (Fig. 2). Major vibriosis epizootics began during the last week of August about 6 wk after the fish were transferred to salt water and continued through September. Ambient water temperature did not influence the timing of the first vibriosis epizootic, but the beginning of the second epizootic was influenced by increasing water temperature (Fig. 2). A few fish were lost due to furunculosis in July and August. Chronic losses due to kidney disease were observed throughout the experiment, but never reached epizootic proportions.

Chinook salmon showed a high level of protection when immunized by HI of *V. anguillarum–A. salmonicida* bacterins. The control group of 495 fish sustained 71.3% mortality in salt water during the experiment, compared with a 16.7% mortality in the 493 fish hyperosmotic infiltrated with *V. anguillarum–A. salmonicida*. *V. anguillarum* was isolated from 74 of 76 of the moribund chinook examined.

The production of serum agglutinating antibody was used as an index to the potency of vaccination treatments. The *V. anguillarum* serum agglutinin titer was monitored over time in all treatment groups (Fig. 3). In all coho treatment groups the titer of *V. anguillarum* antibodies was low shortly after vaccination. Within 5 wk, however, serum antibody increased rapidly in HI *V. anguillarum*, injected *V. anguillarum*, and the injected *V. anguillarum–A. salmonicida* groups. The antibody titer was slightly higher in the HI *V. anguillarum* group than in the injected *V. anguillarum* or injected *V. anguillarum–A. salmonicida* groups on the second, third, and fourth sampling dates. The levels of serum agglutinins in the bivalent injected *V. anguillarum–A. salmonicida* treatment group were closely comparable with those in the injected *V. anguillarum* treatment. Antibody titers in all the *V. anguillarum* vaccinated groups tended to stabilize at a mean titer of about 512 after mid-August. In contrast, the increase in antibody titer was slow until late August in groups that were not immunized against *V. anguillarum* (injected control, injected *A. salmonicida*, untreated control, and HI control). After the first major vibriosis epizootic in late August (Fig. 2), antibody titers increased in the groups that had not been immunized against *V. anguillarum*.

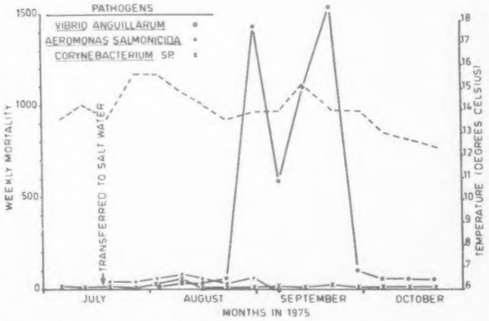

FIG. 2. Causes of coho saltwater pen rearing mortality and 2.0-m ambient water temperature. Data were extrapolated from examination of 473 moribund fish to the total mortality.

The titers of *V. anguillarum* serum agglutinins in two groups of chinook salmon tended to be lower than in the coho salmon tested. Titers in the control group were low (mean below 4, except on the last sampling date) throughout the experiment. The titer of the hyperosmotic infiltrated *V. anguillarum–A. salmonicida* group slowly increased to a mean of about 32 during August; later in September, after the vibriosis epizootic, the mean titer increased to 54.

Only the coho salmon groups immunized with *A. salmonicida* bacterins developed significant levels of serum antibody specific for *A. salmonicida* (Fig. 3). The levels of serum *A. salmonicida* agglutinins were not significantly different (analysis of variance, $\propto = 0.05$) between the injected *A. salmonicida* and the injected *V. anguillarum–A. salmonicida* treatments. The control and *V. anguillarum*-vaccinated groups did not develop high titers of *A. salmonicida* agglutinins.

The growth rate of the coho salmon was not significantly different between replicates (*t*-test, $\propto = 0.05$), nor between treatments (analysis of covariance, $\propto = 0.05$).

Discussion

Hyperosmotic infiltration of *V. anguillarum* bacterins prevented mortality due to vibriosis better than any of the other treatments tested. Since injected vaccine treatments are proven methods of immunizing against vibriosis (Antipa 1976), these results are encouraging. A high degree of protection against vibriosis was demonstrated with both methods, but treatment mortailty was less with the HI method than with the injection method. The primary advantages of the HI method are faster administration of treatment (4–5 times faster than injection) and less dependency on the size of fish at vaccination which allows for greater flexibility in applying this technique to fish culture.

The bivalent *V. anguillarum–A. salmonicida* vaccines, applied by HI in chinook salmon and

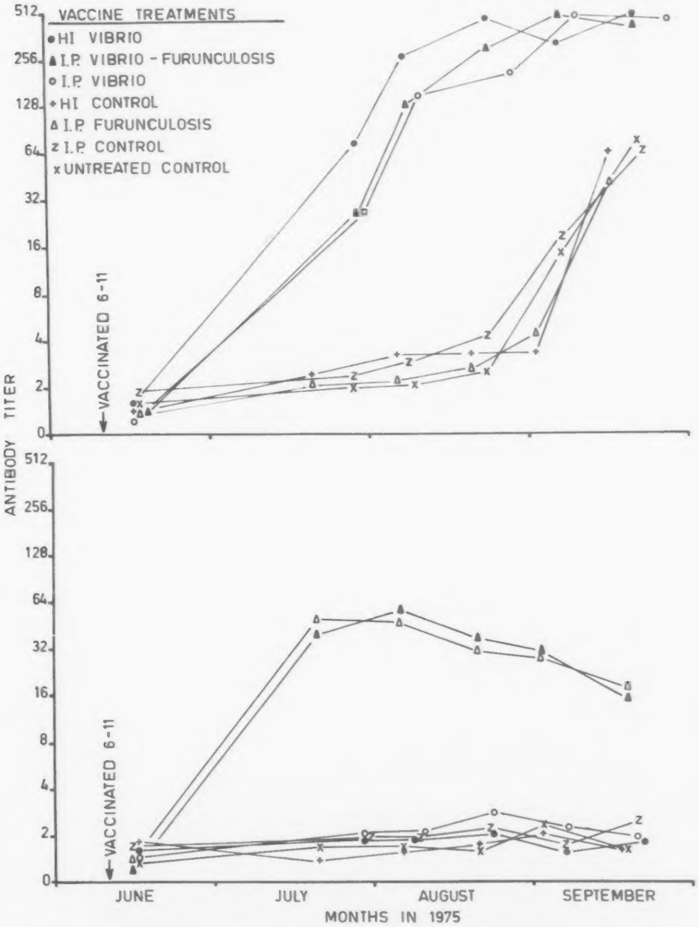

FIG. 3. *Upper*: mean humoral *Vibrio anguillarum* agglutinin titer of coho salmon over time. Each point represents the mean of two 10-fish samples from replicate pens. *Lower*: mean humoral *Aeromonas salmonicida* agglutinin titer of coho salmon over time. Each point represents the mean of a 10-fish sample.

by injection in coho salmon, provided adequate protection against vibriosis, and the serum antibody titers indicated no inhibition of the immune response to either antigen. The lack of a significant furunculosis epizootic prevented the assessment of the degree of protection conferred by the bivalent vaccine against furunculosis.

The role of humoral antibody in the prevention of natural vibriosis and furunculosis infections is not completely known (Antipa 1976; Harrell et al. 1975). In this experiment production of *V. anguillarum* serum agglutinins was similar in fish treated by injection and by hyperosmotic infiltration, and titers of humoral *A. salmonicida* agglutinins were significantly increased by injection. Whether serum agglutinin levels are a reliable index of the degree of disease immunity is a topic that warrants further investigation.

On the basis of the results of Fender (1976), Amend and Fender (1976), and Croy and Amend (unpublished data), the vacuum procedures used in the tests reported here may not have contributed to the efficacy or potency of the hyperosmotic infiltrated bacterins. However, the test was not designed to evaluate this parameter, and therefore the value of the vacuum step is equivocal. Although the hyperosmotic infiltration method provided protection against vibriosis equal to or better than that provided by injection, more tests are needed to determine the best delivery method.

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Chemical and Sensory Changes in Round Inshore Male Capelin (*Mallotus villosus*) During Prolonged Storage for 24 Months at – 23°C

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SHAW, D. H., AND J. R. BOTTA. 1977. Chemical and sensory changes in round inshore male capelin (*Mallotus villosus*) during prolonged storage for 24 months at –23°C. J. Fish. Res. Board Can. 34: 209–214.

Male capelin (*Mallotus villosus*) from a spawning inshore stock were held whole in block frozen form at –23°C without the benefit of special protective treatments. The results indicated an exceptional storage life of the samples, with no development of oxidative rancidity as monitored by organoleptic analysis, although TBA data did indicate this possibility. For many variables, the major chemical and sensory changes appeared to occur as a result of freezing and thawing, rather than as a result of frozen storage; changes were fastest during the first 6 wk. Because of the high sensory scores, it was difficult to correlate most chemical tests and organoleptic changes, though the relationship of texture scores to EPN and FFA values was significant ($P \leq 0.05$). Sensory analyses showed that, after 2 yr, the samples were still fully acceptable.

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Des capelans mâles provenant d'un stock côtier reproducteur ont été conservés ronds en blocs congelés à –23 C sans traitement protecteur spécial. Les résultats indiquent une durée de conservation exceptionnelle des échantillons, sans qu'il se développe de rancidité par oxydation, suivie de façon continue par analyse organoleptique, bien que les données de tests DBA en indiquent la possibilité. Pour plusieurs variables, les principaux changements chimiques et sensoriels semblent résulter de la congélation et de la décongélation plutôt que de l'entreposage congelé; les changements se produisent le plus rapidement durant les 6 premières semaines. A cause des cotes élevées des analyses organoleptiques, il est difficile d'établir une relation entre la plupart des essais chimiques et les changements organoleptiques, bien que la relation entre les cotes de texture et les valeurs de EPN et de FFA soit significative ($P \leq 0.05$). Les analyses organoleptiques démontrent qu'après 2 ans, les échantillons sont encore tout à fait acceptables.

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WE have previously reported the storage characteristics of fresh inshore male capelin (*Mallotus villosus*) when preserved by either ice (Shaw and Botta 1975a) or refrigerated sea water (Shaw and Botta 1975b). As the annual "capelin run" in Newfoundland is of short duration and coincides with the beginning of the more valuable trap-cod season, any method is of value that will satisfactorily preserve whole capelin for periods of from 1 mo to 1 yr prior to the processing stage. Socioeconomic and climatic conditions in Newfoundland ensure that the plants processing fish from the inshore fishery are generally operative only between May and November. The handling of capelin, particularly if they are to be utilized as a canned product, is labor intensive, and once markets are fully developed, it would be more efficient to handle the more traditional inshore species during the normal season and the capelin at a later date when plants would normally be closed. Present technology indicates that freezing is the best method of preservation. With capelin, frozen storage prior to processing would have to be of round fish to avoid the time-consuming heading and gutting operations.

Various authors, notably Jangaard (1974), have commented on the excellent frozen storage quality of capelin; in Newfoundland at least, such a belief is widely held and by all accounts justified. However, Jangaard (1974) does not refer to any critical scientific assessment of the general chemical and organoleptic changes taking place, nor have we been able to find such a study in the literature. Long-term frozen storage of small fatty species has not generally been too successful;

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chub mackerel (*Scomber japonicus*) (FAO 1971) and Atlantic mackerel (*Scomber scombrus*) (Pottinger 1951; Dingle 1976) have been shown to develop rancidity when held in cold storage for longer than 6 mo, and few fatty fish have the potential for frozen storage displayed by most lean fish.

This paper examines changes in chemical indices in frozen inshore male capelin, and assesses the degree to which changes in these indices (particularly those concerned with rancidity development) influence the organoleptic characteristics of this species. The product discussed in this paper will in practice be an intermediate commercial product, most of which will be further processed.

Materials and Methods

Inshore capelin were caught by box-seine in late June approximately 3 km offshore in Conception Bay, Newfoundland. The catch (1175 kg) was landed 3 h later when the average temperature of the fish had reached 5°C. Male capelin were sorted from females and stored in ice for a further 12 h before being packed in wax coated cartons (6.8 kg/box) and plate frozen. The frozen blocks were then stored in the carton at −23°C for the duration of the experiment, and were neither glazed nor overwrapped. At each sampling time, two blocks were thawed in running tap water and the separated fish were headed, gutted and washed, before being repacked in 454 g waxed cartons (approximately 12 fish/carton). In general, six cartons were prepared at each period, three each for subsequent sensory and chemical analyses, respectively. The samples were held at −40°C (for up to 6 mo) until analyzed.

Sensory analyses for taste, texture and overall acceptability were according to a scheme previously used for iced capelin (Shaw and Botta 1975a). Most chemical values — lipid, free fatty acid (FFA), peroxide, thiobarbituric acid value (TBA), hypoxanthine, moisture, and pH — were analyzed by methods annotated or described in the same paper. Trimethylamine (TMA) was measured by the method of Tozawa et al. (1971) and dimethylamine (DMA) by the method of Dyer and Mounsey (1945). Thaw drip was determined by a method suggested by Dyer et al. (1962). Extractable protein nitrogen (EPN) was determined by the procedure of Dyer et al. (1950) modified, because of an inherent interference causing cloudiness during the Biuret determination of protein, as follows: crude protein was determined by micro-Kjeldahl digestion, distillation, and titration of a 3-ml aliquot of the extract; nonprotein nitrogen was determined in similar manner on a 50-ml aliquot of the extract precipitated with trichloroacetic acid (2.5 g) to remove the protein; and extractable protein nitrogen was expressed as the difference between these two values.

Bivariant linear regression analysis was used on the means of the variables to determine whether correlation existed between taste and texture and any of the chemical values. However, correlations only indicate an association of variables not a definite cause and effect relationship. Results presented graphically include the mean and the standard deviation from the mean. We have previously commented on the variability of inshore spawning capelin, and the effect of this variation on results (Shaw and Botta 1975a).

Results and Discussion

CHANGES ASSOCIATED WITH LIPID

Analytical values for lipid varied slightly (Table 1), but the grand mean for the 13 sampling periods was 2.85%, a figure within the limits for fresh capelin (MacCallum et al. 1969). Even though these fish were lipid depleted due to the spawning condition, the value of about 3% is considerably higher than that usually found in lean fish (Sidwell et al. 1974), and capelin have the potential to develop rancidity during later stages of storage; we have previously detected incipient rancidity in capelin with an original lipid content of 1.4% (Shaw and Botta 1975b).

The pattern of FFA development is unusual (Fig. 1), as there appears to be a general decrease in FFA during the first 8 mo followed by the more normal increase, due to hydrolysis of the lipid, in the storage period between the 8th mo and 2 yr (this pattern is not characteristic of pelagic species). The final high FFA concentration confirms results previously reported for male capelin (Ackman et al. 1969). Baltic herring (*Clupea harengus var. membranus*) (Bosund and Ganrot 1969) show the storage pattern of lipid hydrolysis considered normal for fatty fish; increasing FFA reached a maximum at 9 wk during a storage period of 13 wk.

The potential for various lipid changes during frozen storage may be assessed by an analysis of the component fatty acids. The presence of substantial quantities of polyunsaturated acids would indicate a probability of serious oxidative rancidity during storage. The lipids of whole capelin have been shown to contain an average of 20% saturated, 60% monounsaturated, and 20% polyunsaturated acids (mostly $20{:}5\ \omega 3$ and $22{:}6\ \omega 3$) (Ackman et al. 1969). The values for polyunsaturated fatty acids are essentially the same as those reported for Atlantic herring (*Clupea harengus harengus*) oil (Ackman and Eaton 1966), but lower than the 33% reported in Pacific sardine (*Sardinops sagax*) oil (Ackman and Sipos 1964). European herring oils have only 15% polyunsaturated acids (Dunn and Robson 1965) and should be less susceptible to oxidation, barring the interaction of other chemical parameters,

TABLE 1. Changes in body lipid (grams per 100 grams fish), peroxide value (milliequivalents $Na_2S_2O_3$ per 100 grams fat), and TBA value (milligrams malonaldehyde per 1000 grams fish) during storage of capelin (*Mallotus villosus*) at −23°C. All analyses were performed on headed and gutted fish.

Storage time (*mo*)	Lipid	Peroxide	TBA
0.5	1.3	11.24	2.17
1.5	2.5	2.44	3.89
2	3.2	5.25	5.42
3	2.7	6.65	6.00
4	2.5	6.38	5.58
6	3.0	4.13	5.38
8	3.5	4.80	7.39
10	3.2	4.88	4.96
12	3.1	6.34	5.11
15	3.6	3.01	4.09
18	2.6	12.18	7.37
21	3.5	10.82	6.03
24	2.4	9.68	5.59

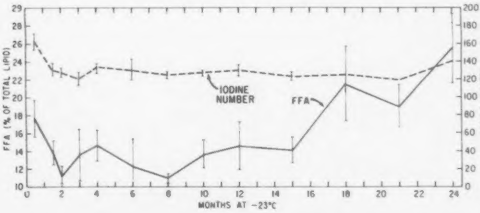

FIG. 1. Changes in FFA and iodine number in capelin (*Mallotus villosus*) stored at −23°C. To avoid confusion, standard deviations for the last three points of the iodine number curve are not shown in the figure, the values were as follows: 18 mo (125.5 ± 12.4); 21 mo (119 ± 14.4); 24 mo (140.2 ± 7.1).

than capelin oils. Oxidative rancidity has been recorded as a serious problem in chub mackerel (Keay et al. 1972), a species in which polyunsaturated fatty acids constitute 30.4% of the flesh lipid, with 40% of these polyunsaturated acids having five or six double bonds (Keay et al. 1972). Because a high degree of polyunsaturation of the fatty acids is an important condition for the rate of development of oxidative rancidity, we would expect capelin to show storage characteristics similar to herring. This similarity of degree of polyunsaturation is confirmed by an average iodine value of 130 reported in this study, compared with 128 for Atlantic herring (Ackman 1966). The small drop in iodine value during the initial stages of storage (Fig. 1) has also been observed in an investigation on chub mackerel when stored at −30°C (Keay et al. 1972). When this species was stored at −14°C however, the iodine number fell regularly from 158 to 110 during 90 days (Keay et al. 1972).

Changes in peroxide value and TBA number (Table 1) were erratic. Though there was a slight overall increase in TBA number from an intial value of 2.17 to a final value at 24 mo of 5.59, the values did not correlate with changes in taste. However, the final value is considerably higher than 2, regarded as the probable threshold value for organoleptic detection (Connell 1975). Other authors have also reported similar disagreement between taste panel results and TBA value (Watts 1961; Mendelsohn and Peters 1963).

MISCELLANEOUS CHEMICAL CHANGES

During the course of storage, EPN decreased from 1.4343 g/100 g fish after 2 wk storage to 0.5433 g/100 g fish (Fig. 2). The decrease in EPN correlated significantly with the increase in FFA ($r = -0.5944$, $n = 13$, $P \leqslant 0.05$). Thaw drip (Table 2) did not change significantly and showed no statistical relationship with textural changes or EPN. The latter correlation had previously been demonstrated in *Scomber japonicus* (Nishimoto 1962) stored at −20°C. The quantities of DMA formed during frozen storage of capelin (Table 2) are small when compared with cod (*Gadus morhua*), pollock (*Pollachius virens*), silver hake (*Merluccius bilinearis*) and Pacific hake (*Merluccius productus*) (Castell et al. 1970, 1971; Babbitt et al. 1972). As formation of formaldehyde is equimolar and concommitant with the formation of DMA, the effect of formaldehyde on insolubilization of protein (Castell et al. 1973) is presumably only a minor factor in the decrease in EPN in frozen stored (−23°C) capelin. Although DMA does not appear to increase significantly in this species and has no significant correlation with texture, the amount

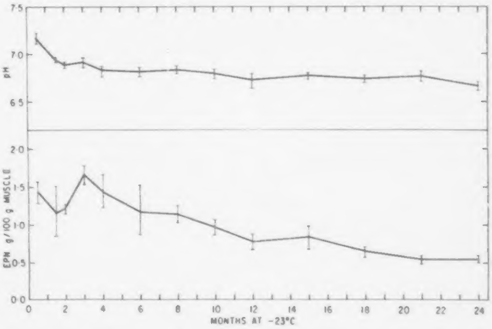

FIG. 2. Changes in pH and EPN in capelin stored at −23°C.

TABLE 2. Changes in hypoxanthine (milligrams per 100 grams fish), DMA (milligrams DMA-N per 100 grams fish), TMA (milligrams TMA-N per 100 grams fish), moisture (percent), and thaw drip (grams per 100 grams fish) during storage of capelin at −23°C. All analyses were performed on headed and gutted fish. ND = not determined.

Storage time (*mo*)	Hypoxanthine	DMA	TMA	Moisture	Thaw drip
0.5	12.1	0.093	0.168	85.1	17.17
1.5	16.0	0.103	0.240	81.4	18.67
2	14.3	0.237	0.488	81.4	14.72
3	16.5	0.200	0.450	81.7	14.04
4	15.0	0.322	0.498	81.3	17.40
6	14.6	ND	ND	81.6	11.36
8	17.4	0.448	0.536	82.3	12.56
10	17.6	0.568	0.662	80.9	11.53
12	20.8	0.574	0.710	80.8	16.82
15	16.1	0.572	0.751	82.8	19.57
18	18.2	0.746	0.934	80.0	14.72
21	19.1	0.551	0.808	81.3	12.61
24	20.4	0.691	1.039	82.1	11.51

formed (and thus that of an equimolar quantity of formaldehyde) correlates significantly with EPN ($r = -0.8413$, $n = 12$, $P \leqslant 0.01$). TMA development is also minimal (Table 2) and the final value of 1.039 mg TMA-N/100 g fish denotes excellent quality. Changes in TMA correlated significantly with taste ($P \leqslant 0.01$) and texture ($P \leqslant 0.05$) but were meaningless as indicators of quality because of the low values. Even though TMA is at a low level at the end of the storage period, it has increased almost sevenfold, the same increase as that of DMA. As the Tozawa et al. (1971) method was used to measure TMA, we do not regard the apparent increase in this value as an artifact but rather as either the product of very slow bacterial degradation or the action of the reducing enzyme found in the red muscle of pelagic fish (Castell 1970).

During the investigation, pH (Fig. 2) fell from a high of 7.17 at 2 wk to a low of 6.66 at 2 yr. A lowering of pH during storage is more normally associated with changes (lactate formation) occurring at ice temperatures. A decrease in pH due to lactate during the first 3 mo of storage at −20°C has however been demonstrated in lingcod (*Ophiodon elongatus*) (Tomlinson et al. 1963). The pH has been regarded as related to textural changes at temperatures below −15°C (Gould and Peters 1971), but our results indicate no significant correlation of these two variables. There is no indication of moisture loss other than a slight dehydration presumably due to storage (Table 2). Our methods of measuring moisture, however, would not have distinguished a decrease in water-binding capacity of the muscle during cold storage. The thaw-drip values indicate that water binding is not decreasing to any extent, and certainly not in line with changing pH values.

Hypoxanthine was produced during the experiment (Table 2) increasing erratically from an initial value of 12.1 mg/100 g muscle to a final value of 20.4 mg/100 g muscle. Because these changes did not significantly correlate with taste scores, this variable was not a good indicator of quality in this study. The slow accumulation of hypoxanthine however confirms observations on Cape hake (*Merluccius capensis*) made by Burt and Simmonds (1969) and is different from similar studies on cod, where hypoxanthine does not accumulate during frozen storage at −20 or −30°C (Jones 1963).

SENSORY ANALYSES

The generally accepted belief that inshore capelin keep well during frozen storage is borne out by the results of organoleptic analysis (Fig. 3). During the experiment, texture scores fell from a high of 92.0 at 0 time to 68.25 at 2 yr. Texture remained relatively unchanged after an initial drop over the first 2 wk, presumably be-

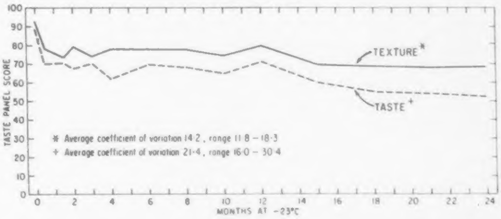

FIG. 3. Sensory analysis of capelin stored at −23°C.

cause freezing and thawing caused the textural changes, rather than time of frozen storage. At the conclusion of sampling, texture was still soft and non-chewy with no obvious gaping. Visible changes had occurred only in the skin, where the silver sheen had changed to a dull light grey, and in the muscle, where a slight browning was observed. Changes in EPN with increasing storage time show a significant ($P \leqslant 0.05$) correlation with texture, a relationship that is usually present during frozen storage, particularly with lean fish (Olley et al. 1967). We expected to observe a greater change in the texture, however, because of the amount of EPN lost during the storage time. Of all the chemical measurements, none correlated with texture at the 1% level or less, and only EPN, TMA, and FFA correlated at the 5% level; all other chemical changes did not appear to bear any relation to texture. Because TMA has not previously been shown to affect texture and because the capelin developed a final value of only 1.04 mg TMA-N/100 g fish, the statistical association is probably not real. The initial pH of the muscle (7.17 at 2 wk) and its effect on texture agrees with a previous study on cod (Kelly 1969). The lack of correlation between dimethylamine production and texture add capelin to the list given by Mills (1975) of species in which dimethylamine is not a good indicator of frozen-storage history.

Free fatty acid production has on occasion been shown to affect the texture of fish significantly (Peters et al. 1968; Shaw and Botta 1975b), and our observations indicate that this relationship holds true for male capelin stored at −23°C. Textural changes in frozen male inshore capelin seem to be related only to EPN and FFA, thus extending the use of these two indicators to at least one small pelagic species.

Changes in taste, although more marked than textural changes, were still relatively small. Trimethylamine and DMA showed a highly significant correlation when compared with taste ($P \leqslant 0.01$) but it is unlikely that this had any meaning in the organoleptic sense, as both metabolites were present in quantities not regarded as detectable. A significant ($P \leqslant 0.01$) relationship between taste and EPN is also fortuitous, as changes in protein solubility have not been reported to affect taste. On the other hand, FFA and taste changed significantly ($P \leqslant 0.01$) with respect to each other, and an oily taste reported by the panel in later samples may be the result of detecting FFA levels of 25%. Rancidity was, however, never indicated during sensory analyses, and this observation confirmed the minimal change, during 24 mo, of a rough indication of chemical rancidity (peroxide value). The taste reached a low level of 52 during the course of the experiment, and the development of the oily taste was the only negative taste feature reported by the sensory panel. Although given the opportunity to comment on odor, the panel found no objectionable odors during the storage period; the characteristic "capelin odor" was present, however, throughout the experiment.

Overall acceptability was measured, but so closely paralleled the taste scores that individual results are not presented in this paper. In general, the organoleptic quality of frozen inshore capelin after 24 mo at −23°C is very good, and this species appears to have a frozen storage life approximately 4 times that of other small pelagic species.

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Heinz Body Hemolytic Anemia in Coho Salmon (*Oncorhynchus kisutch*) Exposed to Chlorinated Wastewater

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BUCKLEY, J. A. 1976. Heinz body hemolytic anemia in coho salmon (*Oncorhynchus kisutch*) exposed to chlorinated wastewater. J. Fish. Res. Board Can. 34: 215–224.

Fingerling coho salmon (*Oncorhynchus kisutch*) were exposed for 12 wk to various concentrations of treated wastewater containing total residual chlorine ($TRCl_2$) with riverwater diluent under continuous flow conditions. Levels of $TRCl_2$ averaging 0.003–0.05 mg/ℓ resulted in mild to pronounced symptoms of hemolytic anemia, including an increase in numbers of circulating immature erythrocytes, pathological changes in erythrocytes, and a reduction in packed cell volume and hemoglobin levels. In addition, Heinz bodies were formed at the 0.012 and 0.05 mg/ℓ $TRCl_2$ levels. The hematologic changes were attributed to the oxidative nature of $TRCl_2$.

BUCKLEY, J. A. 1976. Heinz body hemolytic anemia in coho salmon (*Oncorhynchus kisutch*) exposed to chlorinated wastewater. J. Fish. Res. Board Can. 34: 215–224.

Des fingerlings de saumon coho (*Oncorhynchus kisutch*) ont été exposés pendant 12 semaines à diverses concentrations d'eaux usées traitées contenant du chlore résiduaire total ($TRCl_2$), avec de l'eau de rivière comme diluant et dans des conditions de débit continu. Des niveaux de $TRCl_2$ variant en moyenne de 0.003 à 0.05 mg/ℓ causent des symptômes allant de bénins à prononcés d'anémie hémolytique, y compris une augmentation du nombre d'érythrocytes immatures en circulation, des changements pathologiques dans les érythrocytes et une diminution du volume de cellules de pack et des niveaux d'hémoglobine. De plus, il s'est formé des corps de Heinz aux niveaux de $TRCl_2$ de 0.012 et de 0.05. On attribue à la nature oxydante de $TRCl_2$ ces changements hématologiques.

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ATTENTION has focused on the danger to aquatic organisms in receiving waters from the residual chlorine content of sewage treatment plant effluent (Brungs 1973; Tsai 1973). Combined residual chlorine (chloramine) is formed when chlorine combines with ammonia in the wastewater. Compared with free residual chlorine under laboratory conditions, combined residual chlorine is slightly less toxic (Merkens 1958; Rosenberger 1971) but is more persistent in the receiving waters (Rich 1963; Chambers 1971). Some of the hematologic effects of chloramines on humans and fish have been reported by Eaton et al. (1973). However, blood changes resulting in anemia (Smith et al. 1971; Smith 1968; Whitmore 1965) and elevated numbers of circulating immature erythrocytes (Whitmore 1965; McLeay 1973) may be induced by a wide variety of toxic materials. The general nature of these stress responses indicates that measurement of fish blood characteristics, including erythrocyte morphology, may provide a sensitive means of early warning for some water quality changes adverse to fish life, particularly those resulting from residual chlorine (Buckley et al. 1976). The reports of Whitmore (1965), Eaton et al. (1973) and Buckley et al. (1976) provided much of the rationale for methods used in the present study in which some of the sublethal effects of combined residual chlorine on the blood of coho salmon and the range of concentrations evoking sublethal stress were investigated under laboratory conditions.

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Materials and Methods

STUDY SITE

The study was conducted at the Municipality of Metropolitan Seattle's Renton Treatment Plant, where an average of 111,000 m³/day of domestic sewage is given secondary treatment, chlorinated for disinfection, and subsequently partly dechlorinated with SO_2 prior to discharge into the Green–Duwamish River. The river and its estuary contain approximately 25 species of fish, in addition to large runs of salmon, primarily from hatchery stock.

FISH

Fingerling coho salmon (4 g avg. wet wt) were obtained from the Washington State Department of

Fisheries Green River hatchery and divided into 12 groups of 124 fish each. Oregon Moist Pellets were fed twice daily on weekdays (3% of body wt/day) and once on Saturday or Sunday (2% of body wt/day). Feed was withheld 24 h prior to blood and weight sampling.

External and internal examination of the 590 fish sampled for blood revealed no signs of disease.

The average wet weight of the groups was measured at weeks 0, 4, 8, 12, 16; and the fork length, wet weight (with surface moisture removed), and dry weight (constant weight in 102°C oven) of each fish were taken at the end of the test. Differences between control and test groups were tested for significance by analysis of variance.

EXPERIMENTAL DESIGN

Acclimation (8 days) and testing were conducted in 400-ℓ, circular fiberglass tanks provided by polyvinyl chloride pipe with a continuous flow of effluent diluted with river water. Chlorinated effluent (CE) and partly dechlorinated effluent (PDE) were taken by metal-free and stainless-steel pumps, respectively, retained for a period approximating travel time to the river outfall, and diluted with river water obtained by a cast-iron pump. Flow rate to each tank was 5 ℓ/min (95% replacement of tank content in 4 h), with a maximum measured error of less than 2% of the nominal tank effluent concentration. The tanks and diluter were cleaned routinely.

The experiment consisted of two parts, during which duplicate groups of fish underwent: 1) a 12-wk exposure to either the control (0%) or one of the five test solutions (either 0.2, 1.0, or 3.7% CE or 3.8 or 10% PDE), and 2) a 4-wk recovery period in 100% river water.

BLOOD SAMPLING AND ANALYSIS

Blood was sampled from 10 fish taken randomly from each group weekly or biweekly between 0 and 12 wk and at 16 wk. The fish were anesthetized in a continuously aerated solution of MS-222® (80 mg/ℓ of river water), and blood was taken from the severed caudal preduncle. Smears were prepared immediately from the unheparinized blood, air dried, fixed in absolute methanol, and stained with buffered (pH 6.6) Giemsa.

Five hundred erythrocytes from each smear were classified for maturity under an oil-immersion microscope using morphology and staining properties as criteria. Concurrently the percentage of leukocytes was estimated. Methyl violet (Dacie and Lewis 1968) and brilliant cresyl blue (Cartwright 1968) were used to stain Heinz bodies. Smears were not fixed or counterstained. Total hemoglobin (Hb) concentration was determined by the Cyanmethemoglobin method, packed cell volume (PCV) by the microhematocrit method, plasma total dissolved solids (TDS) with a refractometer, and total bilirubin by the American Monitor modification of the method of Nosslin (1960). Significant differences in blood measurements were determined by the nonparametric test of Lord (1947).

WATER QUALITY DETERMINATION

Midtank and effluent samples were analyzed according to the American Public Health Association (1971), except ammonia and nitrite, which were measured by the method of Zadorojny et al. (1973) and Strickland and Parsons (1968), respectively (Table 1). The heavy metals content of acidified samples was measured on a IL453 atomic adsorption spectrophotometer. In addition to the heavy metals shown in Table 1, Cr and Pb were consistently below the detection limits of 5 and 20 μg/ℓ, respectively. Because the complexing materials in sewage effluent and river water reduce the toxicity of Cu (Brown et al. 1974) and probably other metals (Ramamoorthy and Kushner 1975), the metals content of the test solutions was considered to have little influence in this investigation. The pH ranged from 6.92 to 7.32 and differed only slightly between tanks at any one time. The tank surface water temperatures averaged 14.8 C and ranged from 8.9 to 20.2 over the test period, which approximated the temperature regime of ambient river water. The total residual chlorine ($TRCl_2$) concentrations in the tanks (Table 1) were calculated from data registered on a 24-h/day strip-chart recorder, which monitored both the chlorinated and partly dechlorinated effluents (Fig. 1 D, E). The calculated values were corrected by measurements from in-tank grab samples analyzed by a modified amperometric titration procedure with a detection limit of 0.01 mg/ℓ. Because of high ammonia content (average 14.5 mg/ℓ total NH_3-N), the amount of free residual chlorine in the effluent was negligible and the measured $TRCl_2$ consisted of combined residual chlorine, probably in the monochloramine form. Nitrite levels in the undiluted chlorinated and dechlorinated effluents averaged 0.02 and 0.96 mg/ℓ NO_2-N, with 0.02 and 0.63 SD, respectively. The effluent surfactant concentration, measured as methylene blue-active substances (MBAS), averaged 0.24 mg/ℓ.

Results

Previous tests at this laboratory have established that the toxicity of the effluent is principally determined by the $TRCl_2$ content (Buckley and Matsuda 1973). In the present study, stress to the fish was directly related to the $TRCl_2$ content of the effluent to which they were exposed, as shown by time plots of Hb concentration and percentage of immature erythrocytes (Fig. 1). Furthermore, hematologic changes were correlated with $TRCl_2$ fluctuations over time (Fig. 2, 3).

Exposure to test solutions with average concentrations of $TRCl_2$ from 0.003 to 0.050 mg/ℓ resulted in significant ($P = .05$) reductions in Hb and PCV and a numerical increase in circulating immature erythrocytes. High levels of $TRCl_2$ caused morphological and probably physiological oxidant damage to erythrocytes (Eaton et al. 1973; Grothe and Eaton 1975), resulting in hemolytic anemia. Fish exposed to an average

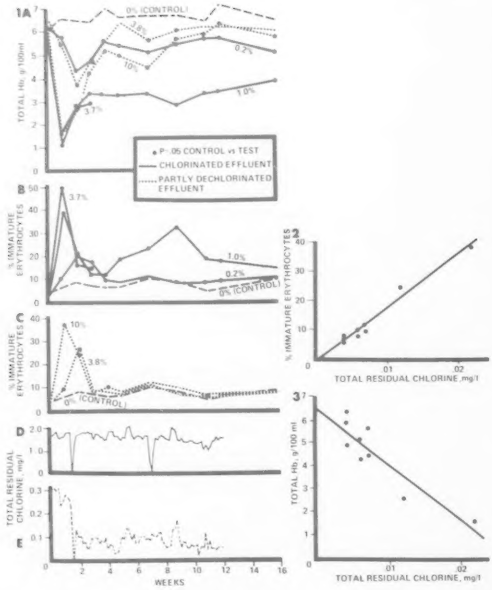
TABLE 1. Water quality of various concentrations of a chlorinated sewage treatment plant effluent with riverwater diluent.

Effluent(%)	Avg.[a]	SD	Maximum	Minimum
	Total residual chlorine[b] *(mg/ℓ)*			
0[c]	.000(84)			
0.2	.003(84)	.001	.004	.001
1.0	.012(84)	.003	.016	.008
3.7	.050(20)	.006	.053	.036
3.8[d]	.003(84)	.002	.009	.001
10[d]	.007(84)	.005	.024	.001
	NH_3-N (un-ionized) (mg/ℓ)			
0[c]	.001(66)	.001	.006	<.001
0.2	.001(66)	.001	.005	<.001
1.0	.001(66)	.001	.005	.001
3.7	.002(26)	.001	.004	<.001
3.8[d]	.003(66)	.001	.007	<.001
10[d]	.006(66)	.002	.018	<.001
	Dissolved oxygen (mg/ℓ)			
0[c]	6.8(61)	1.0	8.5	3.7
0.2	6.8(60)	1.0	8.7	3.6
1.0	6.9(62)	1.0	8.5	3.8
3.7	6.9(18)	.4	7.3	6.2
3.8[d]	6.5(60)	1.0	8.2	3.3
10[d]	6.3(60)	1.0	7.9	3.3
	Total alkalinity (mg/ℓ) as $CaCO_3$			
0[c]	40.6(12)	6.8	48.1	28.9
0.2	40.5(12)	6.5	48.2	28.3
1.0	41.2(12)	6.5	49.0	29.2
3.7	44.3(4)	2.9	47.0	41.5
3.8[d]	43.2(12)	6.9	51.8	29.8
10[d]	47.0(12)	5.6	52.5	36.0
	Metals (μg/ℓ)			
	Cd[a]	Cu[a]	Ni[a]	Zn[a]
0[c]	4(6)	<5(6)	20(6)	16(6)
0.2	<4(6)	5(6)	<20(6)	15(6)
1.0	<4(6)	<5(6)	<20(6)	16(6)
3.7	6(2)	<5(2)	40(2)	36(2)
3.8[d]	<4(6)	5(6)	20(6)	19(6)
10[d]	<4(6)	<5(6)	<20(6)	21(6)

[a] Average of (N) individual tests.
[b] Daily average.
[c] Control.
[d] Partly dechlorinated.

0.012 and 0.050 mg/ℓ TRCl$_2$ (1.0 and 3.7% CE) additionally showed Heinz body inclusions. An excessive number of TRCl$_2$-induced mortalities terminated investigation of the latter group after the 3rd wk. The groups exposed to 0.012, 0.050, and 0.007 mg/ℓ TRCl$_2$ (1.0 and 3.7% CE and 10% PDE), suffered mortalities of 29, 81, and 23%, respectively.

The partial recovery of the Hb and immature erythrocyte levels after the 2nd wk may be accounted for by two mechanisms: 1) decreased hemolysis as a result of elimination of susceptible (probably old) cells and decreased sensitivity of younger cells to oxidants (explanation of a hemolytic process in humans proposed by Dern et al. 1954), and 2) compensatory erythropoiesis with the establishment of a balance between cell destruction and formation resulting in reduced numbers of circulating cells, on the basis of PCV values (Table 2). A temporary malfunction in effluent chlorination control caused a l-h rise in TRCl$_2$ to 0.1 and 0.03 mg/ℓ in 3.7 and 1.0% CE, respectively, which may have contributed to stress during the first 3 wk of the test.



FIGURES 1–3.

FIG. 1. Relationship between (A) average total hemoglobin; (B, C) average percent immature erythrocytes in fingerling coho salmon (*Oncorhynchus kisutch*); and (D, E) total residual chlorine in 100% effluent during period of exposure to various percentages of chlorinated and partly dechlorinated effluent in riverwater diluent (weeks 0–12) and during recovery period in 100% river water (weeks 13–16). FIG. 2. Least-square regression of the effect of weekly average total residual chlorine concentration on percent immature erythrocytes in blood of coho salmon exposed to 10% partly dechlorinated effluent (riverwater diluent) for 12 wk ($r = .99$, $P = .001$). FIG. 3. Least-square regression of the effect of weekly average total residual chlorine concentration on total hemoglobin in coho salmon exposed to 10% partly dechlorinated effluent (riverwater diluent) for 12 wk ($r = -.90$, $P = .005$).

TABLE 2. Average percentages of packed cell volume in coho salmon (*Oncorhynchus kisutch*) exposed to various concentrations of a chlorinated sewage treatment plant effluent with time.

Time (*wk*)	4	5	7	9	11	12	16[a]
0%[b]	39	37	39	38	35	39	35
0.2%	37	37	34[d]	33[d]	35	35[d]	32
1.0%	29[d]	30[d]	30[d]	25[d]	26[d]	27[d]	27[d]
3.8%[c]	36	36	35[d]	35	34	38	35
10%[c]	36[d]	35	33[d]	34[d]	34	35[d]	34

[a] Four weeks after termination of exposure to effluent.
[b] Control.
[c] Partly dechlorinated effluent.
[d] $P < .05$ control vs. % effluent.

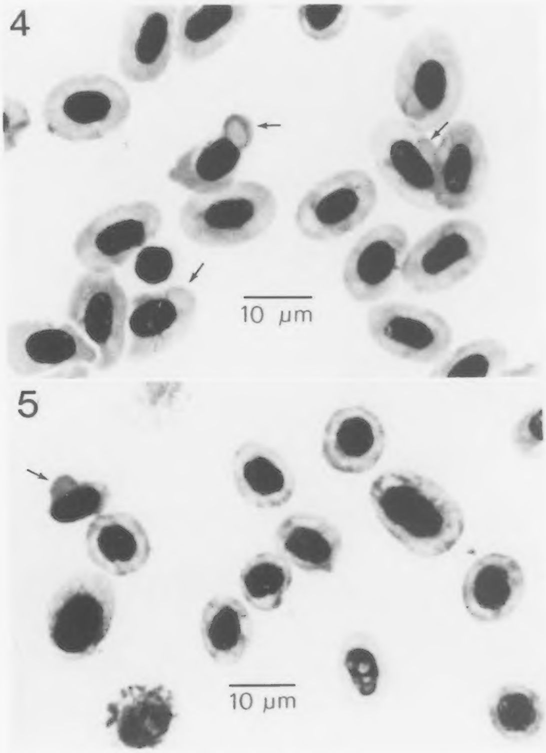
ERYTHROCYTES

The immature erythrocytes consisted of some or all of these developmental stages: pronormoblast and basophilic, polychromatophil, and orthochromatophil normoblasts (nomenclature of Wintrobe 1946). With increasing stress, more primitive forms appeared.

At average concentrations of 0.003 (0.2% CE and 3.8% PDE) and 0.012 (1.0% CE) mg/ℓ $TRCl_2$, the immature cells included mostly orthochromatophils and polychromatophils and a few basophilic normoblasts. During periods of most severe stress poikilocytosis was observed and damaged cells exhibited clumped chromatin with increased interchromatin spaces and a "ragged" appearance of cytoplasmic membranes (Fig. 4). At the 0.012 mg/ℓ $TRCl_2$ level, microcytosis was evident and, at times, apparently mature erythrocytes had gray cytoplasm, probably due to thickening of the cell (based on similar appearance when the cytoplasm of two cells overlap) or cell membrane or other cytoplasmic changes which resulted in altered staining properties. During periods of high stress, dividing cells and cytoplasmic fragments from both mature and immature cells were observed.

Changes in the blood of fish exposed to 0.007 mg/ℓ $TRCl_2$ (10% PDE) were intermediate in severity between those of fish exposed to 0.003 and 0.012 mg/ℓ $TRCl_2$.

Abnormalities at the 0.050 mg/ℓ $TRCl_2$ level (3.7% CE) were similar in nature to those at the 0.012 mg/ℓ $TRCl_2$ level, but fragmentation of erythrocytes, an indication of hemolysis (Dacie 1960), and microcytosis were more severe, and hypochromia was suspected (Fig. 5) although it was obscured by presumed changes in staining properties produced by the intense oxidant stress. Early at both levels spindle-shaped mature erythrocytes appeared. In the 3rd wk of exposure to 0.050 mg/ℓ $TRCl_2$, Heinz body inclusions occurred in the cytoplasm and occasionally in the nuclear region of erythrocytes. They were also seen in various stages of dissociation from the mature, or nearly mature, erythrocytes. The inclusions stained supravitally with methyl violet (Fig. 6) and brilliant cresyl blue (Fig. 7) but slightly or not at all with Giemsa (Fig. 8). Fixation after methyl violet staining resulted in destaining of the bodies. Similar inclusions were observed with Giemsa stain only in fish exposed to 0.012 mg/ℓ $TRCl_2$ (Fig. 4). Spherocytosis, in proportion to stress, was observed; but it was not possible to relate it to $TRCl_2$ toxicity because of



FIGURES 4 and 5.

FIG. 4. Erythrocytes with cytoplasmic deterioration, and Heinz body inclusions (arrows), from coho salmon exposed to an average 0.012 mg/ℓ total residual chlorine (1.0% chlorinated effluent in riverwater diluent) for 12 wk. FIG. 5. Erythrocytes with nuclear and cytoplasmic deterioration from coho salmon exposed to an average 0.05 mg/ℓ total residual chlorine (3.7% chlorinated effluent in riverwater diluent) for 3 wk. A Heinz body inclusion is shown (arrow).

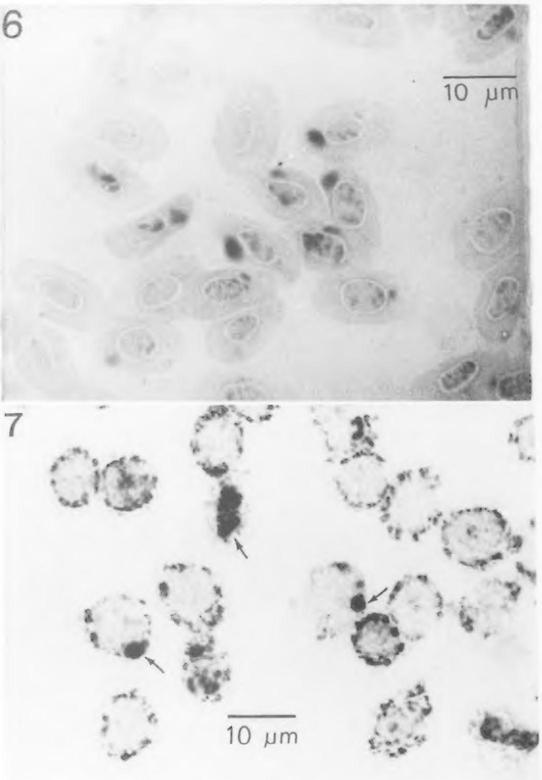
similarities with abnormally developed or stained immature cells.

In contrast to the blood of stressed fish, the blood of control fish had fewer orthochromatophils, infrequent polychromatophils, and fine-grained chromatin (Fig. 9).

As exposure lengthened, Howell–Jolly bodies, common in destructive anemias (Simmons 1968), were observed in the erythrocytes of stressed fish (Fig. 10). During the 16th wk basophilic streaks, evidently the fragmented reticulum, were seen in the otherwise eosinophilic cytoplasm of apparently healthy, mature erythrocytes. They were not restricted to the blood of previously stressed fish. Senile erythrocytes, with nuclei varying from pyknotic to dispersed, and nuclear shadows (Yuki 1960) were apparent in the smears of blood from both test and control fish. Differential counts of leukocytes (lymphocytes + heterophils) did not show a definite trend in relation to effluent concentration.

BLOOD CHEMISTRY

Hemoglobin levels were significantly less in all test fish than in control fish by the end of the 1st wk. After the 3rd wk the average Hb levels in fish exposed to average concentrations of 0.003 and 0.012 mg/ℓ TRCl$_2$ (0.2 and 1.0% CE) were 81 and 47% of control values, respectively.



FIGURES 6 and 7. Stained Heinz bodies in erythrocytes from coho salmon exposed to an average 0.05 mg/ℓ total residual chlorine (3.7% chlorinated effluent in riverwater diluent) for 3 wk.

FIG. 6. Stained with methyl violet; the cells are unfixed and uncounterstained. FIG. 7. Stained with brilliant cresyl blue.

In fish in PDE throughout the test, Hb values generally increased reflecting a gradual decrease in the effluent content of $TRCl_2$.

At the beginning of the 4th wk when sufficient blood was available, PCV showed significant reductions that were proportional to the amount of $TRCl_2$ in the test solutions (Table 2).

Plasma TDS, which has been correlated with plasma protein concentration (Cardwell et al. 1971), was slightly lower in the test fish than in the controls which averaged 7 g/100 ml. The values for test fish did not always reflect the degree of stress shown by other blood measurements.

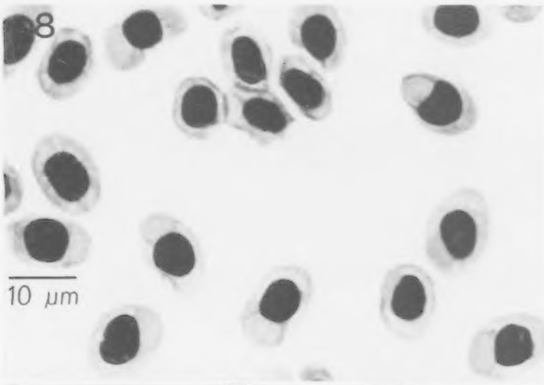
Bilirubin levels (0.05 mg/100 ml) in fish exposed to an average 0.012 mg/ℓ $TRCl_2$ (1.0% CE) for 12 wk were similar to those of control fish. Percent PCV in these test fish, all of which had enlarged spleens, ranged from 2 to 25, compared with 31 to 36 in control fish.

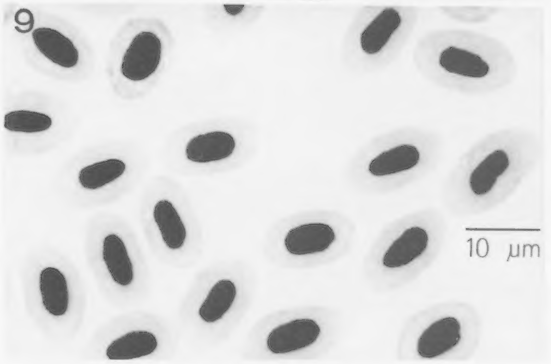
RECOVERY

Four weeks after termination of exposure to $TRCl_2$ Hb and PCV values had decreased slightly, except in those fish that had been exposed to 0.012 mg/ℓ $TRCl_2$ (1.0% CE). Compared to control values, however, little change occurred. Morphologically, the erythrocytes of test fish compared favorably with those of control fish.

WEIGHT AND FEEDING RESPONSE

All groups of fish increased in wet weight, but the groups exposed to PDE had the greatest final





FIGURES 8 and 9.

FIG. 8. Heinz bodies in the cytoplasm of Giemsa-stained erythrocytes of coho salmon exposed to an average 0.05 mg/ℓ total residual chlorine (3.7% chlorinated effluent in riverwater diluent) for 3 wk. FIG. 9. Erythrocytes from coho salmon (control) after 12 wk in 100% river water.

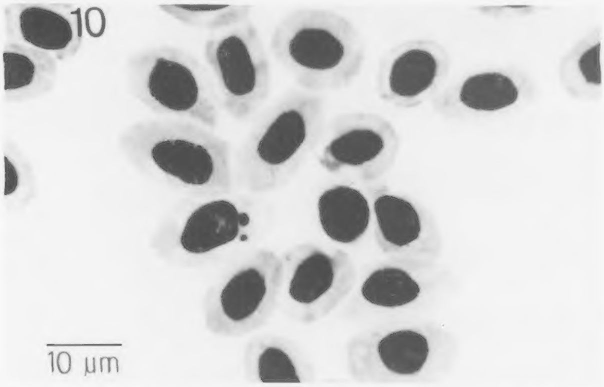


FIG. 10. Howell–Jolly bodies in the cytoplasm of Giemsa-stained erythrocytes of coho salmon exposed to an average 0.05 mg/ℓ total residual chlorine (3.7% chlorinated effluent in riverwater diluent) for 2 wk.

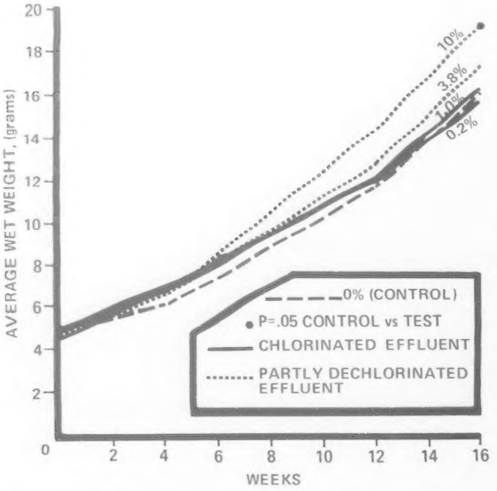


FIG. 11. Changes in average wet weight of coho salmon during period of exposure to various percentages of chlorinated and partly dechlorinated effluent in riverwater diluent (weeks 0–12) and during period of recovery in 100% river water (weeks 13–16).

TABLE 3. Average wet, dry, and body weights (grams) and average fork lengths (centimeters) of coho salmon (*O. kisutch*) after exposure to various percentages of chlorinated effluent (riverwater diluent) for 12 wks and 100% river water for an additional 4 wks.

Effluent (%)	Wet wt	Dry wt	Fluid wt	Fork length	*N*
0[a]	14.9	4.4	10.5	10.7	118
0.2	15.3	4.3	11.0	10.5	127
1.0	15.6	4.1	11.5	10.8	46
3.8[b]	16.4	5.3[c]	11.1	11.1[c]	136
10[b]	18.4[c]	5.7[c]	12.6[c]	11.5[c]	76

[a] Control.
[b] Partly dechlorinated.
[c] $P < .05$.

average weight even though they were anemic for much of the test (Fig. 11). An analysis of the wet and dry weights of individual fish, however, revealed that edema was not a factor (Table 3). The fish in PDE also had significantly greater fork length than the control fish. A comparison of the condition factors (weight/length^3) of the group exposed to 10% PDE and the control group revealed no significant ($P = .05$) difference.

Throughout the study there was a vigorous response to food in all groups except those exposed to 1.0 and 3.7% CE. In these groups, the responses were fair and poor, respectively, but after termination of exposure to the effluent, the surviving fish became as vigorous as those in the other groups.

Discussion

Hemolytic anemia caused by TRCl$_2$ was indicated by a numerical increase in circulating immature erythrocytes, increased destruction of erythrocytes, such as fragmentation (schistocytosis), and general degenerative changes in the erythrocytes, including the formation of Heinz body inclusions. The appearance of Heinz body inclusions in fish exposed to TRCl$_2$ had not been reported previously but was predictable on the basis of several established facts: 1) TRCl$_2$ has a marked effect on the erythrocytes of fish, 2) chloramines can oxidatively denature human Hb into Heinz bodies (Eaton et al. 1973), 3) TRCl$_2$ is a known oxidant, and 4) formation of Heinz bodies is induced in homeotherms by a large number of organic and inorganic redox compounds (Fertman and Fertman 1955). The Heinz bodies were generally larger than those observed in the blood of homeotherms; however, considerable interspecific difference in size (Webster 1949; Fertman and Fertman 1955; Webster et al. 1949), and growth by coalescence (Heinz 1890) have been reported. Heinz body hemolytic anemia in a nonmammalian species has not been reported previously.

In a study of hemolytic anemia in patients undergoing hemodialysis, Eaton et al. (1973) found that chloramines inhibited the metabolism of the hexose monophosphate shunt (HMPS), a glycolytic pathway that is important in protecting the erythrocyte from oxidant damage. Grothe and Eaton (1975) have proposed that this inhibition also occurs in the erythrocytes of fish under similar conditions. In most mammals glutathione, reduced through HMPS activity, plays an important role in the protection of susceptible sulfhydryl groups, including those of globin, from oxidation (Allen and Jandl 1961), and subsequent hemolysis (Fegler 1952). Benesch and Benesch (1954) reported that sulfhydryl groups are critical in the maintenance of the intact erythrocyte. Furthermore, Ball (1933) found that low levels of Ag, an element with a known affinity for sulfhydryl groups, causes lysis of the erythrocytes of fish.

Chlorine may act on the Embden–Meyerhof pathway in erythrocytes, in addition to the HMPS. Green and Stumpf (1946) suggested that chlorine is bactericidal by means of interference with glycolysis. They attributed its action to irreversible inhibition of triosephosphoric dehydrogenase (phosphoglyceraldehyde dehydrogenase) through oxidation of enzyme sulfhydryl groups. Later, Knox et al. (1948) found the aldolase–triosephosphoric dehydrogenase of muscle to be chlorine-sensitive. They also reported that the aldolase of *Escherichia coli*, analogous to that of muscle and yeast (Utter and Werkman 1941), is sensitive to chlorine. It remains to be seen whether $TRCl_2$ acts similarly on the glycolytic enzymes of piscine erythrocytes under the experimental conditions of this investigation.

Methemoglobinemia has been identified in some studies as a basis for the toxicity of chloramine. Eaton et al. (1973) in the study of hemolytic anemia in humans found that chloramines caused methemoglobinemia. In a study of the mechanism of acute toxicity of monochloramine to fathead minnows (*Pimephales promelas*) by Grothe and Eaton (1975), exposure to 1.5 mg/ℓ for 1 h resutled in a methemoglobin (MHb) level of about 30% of total Hb; therefore, methemoglobinemia and the resulting anoxia were considered the basis for the toxicity of monochloramine under the test conditions. In a study of the sublethal effects of $TRCl_2$ (probably in the monochloramine form) on coho salmon, exposure to 0.03 mg/liter resulted in MHb levels of 8.2% of total Hb and 40% mortalities (Buckley et al. 1976). Under those conditions methemoglobinemia was not considered the primary cause of death, although oxidant-included red cell destruction and resultant anemia may have caused the mortalities.

In this study sublethal stress was observed in fish exposed to fluctuating levels of $TRCl_2$ averaging 0.003–0.012 mg/ℓ. Dandy (1972) observed depressed activity in brook trout (*Salvelinus fontinalis*) exposed to residual chlorine concentrations as low as 0.005 mg/ℓ for 7 days and considered this a sublethal level, below which the threshold for depressant response must occur. Merkens (1958) reported that 0.08 mg/ℓ residual chlorine, mostly monochloramine, killed about half of a group of rainbow trout (*Salmo gairdneri*) in 7 days and suggested that 0.004 mg/ℓ may be a safe concentration. Using fish of the same species, Sprague and Drury (1969) found significant avoidance reactions to 0.01 and 1.0 mg/ℓ residual chlorine levels and a preference for an intermediate level of 0.1 mg/ℓ, which was lethal in about 4 days. They surmised that deadened sense organs may have prevented avoidance at this level. In a 21-wk test with fathead minnows, the highest $TRCl_2$ concentration with no significant effect on egg production was 0.0165 mg/ℓ (Arthur and Eaton 1971). Using fish of the same species in diluted chlorinated sewage in a 43-wk test, Arthur et al. (1975) found that an average 0.014 mg/ℓ $TRCl_2$ had no long-term adverse effect, but an average 0.042 mg/ℓ adversely affected survival over the test period. In an earlier test with diluted chlorinated sewage, Zillich (1972) reported sublethal stress to fathead minnows at 0.04 mg/ℓ residual chlorine.

Brungs (1973) recommends that in areas continuously receiving chlorinated wastes, $TRCl_2$ should not exceed 0.002 mg/ℓ for the protection of most organisms. The Environmental Protection Agency (1973) proposes that under continuous exposure conditions, 0.003 mg/ℓ is the maximum acceptable concentration of residual chlorine in fresh water. The results of this study substantiate those criteria.

Acknowledgments

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Surface Area of the Euphausiid *Thysanöessa raschii* and Its Relation to Body Length, Weight, and Respiration[1]

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HARDING, G. C. H. 1977. Surface area of the euphausiid *Thysanöessa raschii* and its relation to body length, weight, and respiration. J. Fish. Res. Board Can. 34: 225–231.

A method is described for estimating the surface area of marine crustaceans. The external surface area of the euphausiid *Thysanöessa raschii* (M. Sars) is proportional to length$^{2.4}$, dry weight$^{0.95}$, and wet weight$^{0.84}$. Oxygen consumption is proportional to wet weight$^{0.82}$, which indicates that respiration should be proportional to respiratory surface area. The implications of this finding regarding the relations of metabolic rate, size, and surface area are discussed in a broader framework by comparing them with similar studies on vertebrates and other invertebrates.

HARDING, G. C. H. 1977. Surface area of the euphausiid *Thysanöessa raschii* and its relation to body length, weight, and respiration. J. Fish. Res. Board Can. 34: 225–231.

L'auteur décrit une méthode permettant d'estimer la superficie du corps des crustacés de mer. La superficie externe de l'euphauside *Thysanöessa raschii* est proportionnelle à la longueur$^{2.4}$, au poids sec$^{0.95}$ et au poids humide$^{0.84}$. La consommation d'oxygène est proportionnelle au poids humide$^{0.82}$, ce qui indique que la respiration devrait être proportionnelle à la surface respiratoire. On analyse dans un contexte plus général les implications de cette découverte concernant les relations entre le taux métabolique, la taille et la superficie en les comparant avec des études semblables effectuées sur des vertébrés et d'autres invertébrés.

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SMALL aquatic crustaceans respire through their body surface. Their exposed surface is similarly important in determining the uptake rate of hydrophobic pollutants such as petroleum hydrocarbons (Lee 1975) and organochlorine compounds (Wildish and Zitko 1971; Ware and Addison 1973). Thus, a consistent method to measure the surface area of marine crustaceans is needed. Equating a crustacean's shape to a simple geometric figure such as a sphere, cylinder, or spheroid is of dubious value since it does not account for the surface area of the appendages with their setae and setules.

The surface area of irregular-shaped tumors has been estimated by quantifying the sodium chloride adsorbed onto or present in the surface film on these tissues, following emersion from a known salt solution (Zeidman 1948). Tumors were precoated with agar to provide a uniform wetting surface and the method calibrated using agar-coated objects with easily calculated surface area.

There are two reasons why Zeidman's technique cannot be directly applied to marine crustaceans. Coating crustaceans with an agar solution sticks the appendages and body together and dipping marine organisms into 0.9% salt solution causes them to swell osmotically and rupture. Zeidman's method is modified here and recalibrated for use with chitinous surfaces. Measured surface area of euphausiids is then related to body length, weight, and respiratory rate.

Materials and Methods

MEASUREMENT OF SURFACE AREA

Pieces of exoskeleton were dissected from the body of the sand shrimp *Crangon septemspinosa* (Say) and the attached internal tissues were meticulously removed. A camera lucida drawing was made of each piece of exoskeleton and its area was determined with a planimeter. This gives an underestimate of the true surface area because it is assumed that the surfaces are two dimensional. Furthermore, the inner surface has a greater area than the outer because it

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[1] Bedford Institute of Oceanography Contribution.

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is impossible to remove all attached tissue material. The planimeter measurement, however, does give a consistent estimate of surface area since the proportion of inner to outer surface remains the same.

The amount of sodium chloride adsorbed onto or in the surface film on pieces of exoskeleton of known surface area, following emersion from a salt solution with the salinity of sea water, was quantified by titration for calibration purposes. Exoskeletons, held with fine forceps, were immersed in a 3.4% sodium chloride solution, removed, and blotted briefly on absorbent tissue. Forceps were rinsed in deionized water and dried before each immersion. Exoskeletons were then immersed in 5 ml of silver nitrate (0.15%) and sodium nitrate (3.25%) solution and shaken gently to displace the resulting silver chloride precipitate. Exoskeletons were alternately dipped as many as 5 times between the chloride and nitrate solutions, depending on the size of the exoskeletons. Repeated dipping in the same nitrate solution was necessary to detect a change in the silver nitrate concentration. The amount of silver nitrate remaining in the solution was determined by titration with 0.15% ammonium thiocyanate in the presence of concentrated nitric acid (1 ml) and ferric ammonium sulfate (0.1–0.2 g). The amount of sodium chloride per dip that reacted with silver nitrate can be calculated from:

$$1.15\ [\text{blank (ml)} - \text{titrated (ml)}]/\text{no. of dips}$$

where the coefficient is the normality of ammonium thiocyanate multiplied by the molecular weight of sodium chloride. This procedure was replicated for each piece of exoskeleton with five nitrate solutions. The first nitrate solution was discarded because the first dip results in lower salt values than succeeding ones, presumably the result of a chemical reaction on the cuticle by the nitrate solution. Thus, four salt determinations were made for each piece of exoskeleton.

Planimeter-derived surface areas were plotted against sodium chloride values and the best least squares fit was found to be linear (Fig. 1).

Euphausiid exoskeleton is thin and flexible making it difficult to manipulate. A few surface area determinations of euphausiid, *Thysanöessa raschii*, exoskeleton are included in Fig. 1 to show that they fall within the range of values obtained for shrimp. The present calibration of cuticular surfaces and Zeidman's calibration of agar-coated surfaces are identical once his axes are interchanged. Both approaches to calculating surface area are, therefore, complementary.

Surface areas of individual euphausiids can now be determined with the salt method, taking care that pockets of salt solution are not retained between the appendages. The possibility exists that salts within the organism could diffuse out into the nitrate solution. Blanks were performed by substituting the chloride solution with a 3.4% sodium nitrate solution. No correction was found necessary.

RESPIRATION RATE

All euphausiids were collected during January and February 1976 in coastal waters of 1–2°C either from Bedford Basin or St. Margaret's Bay, Nova Scotia. Euphausiids were acclimated in temperature-controlled (±0.5°C), flowing-seawater tanks for 1 day before experiments and fed with developmental stages of copepods. Two experimental temperatures were chosen, one to approximate environmental conditions at the time of collection (1.2°C) and the other much higher (5.6°C) but within the seasonal range of temperature experienced by this euphausiid. Euphausiids were placed individually in 30-ml glass-stoppered flasks containing membrane filtered (0.22 µm, Millipore) sea water. Control flasks contained membrane-filtered sea water. Euphausiid and control flasks were incubated for 3–10 h, depending on the size of organism. Dissolved oxygen was measured in euphausiid and control flasks, before and after incubation, from 2-ml aliquots using a Gas Analyzer with an accuracy of ±0.05 mg/ℓ. Euphausiid length, the distance between rostrum and telson, was determined to the nearest 0.5 mm. Euphausiids were weighed alive after brief blotting on absorbent tissue and were then weighed dry to ±0.05 mg after 24 h at 60°C.

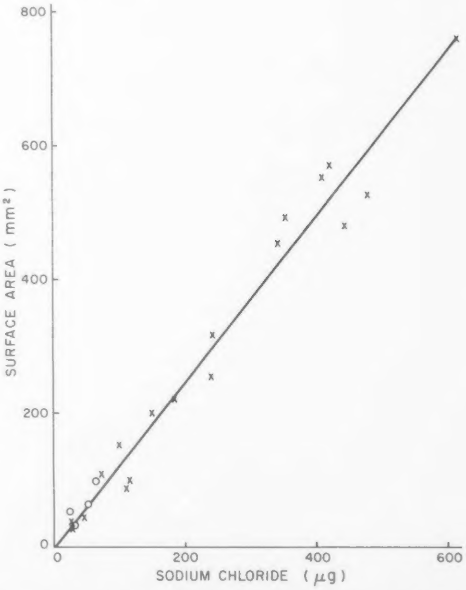

FIG. 1. Calibration curve showing the relationship between the quantity of salt remaining on cuticular surfaces following emersion from a salt solution and their surface area. $S = 1.23x - 1.68$, $r = 0.97$. Exoskeletons used are from *Crangon septemspinosa* (X) and *Thysanöessa raschii* (○).

STATISTICS

The variates to be related in the present paper possess inherent or biological variability and are also subject to errors of measurement. Ricker (1973) has clearly shown that the geometric mean estimate of the functional regression should be employed to describe length–weight and respiration–weight relationships because the variability in the observations is likely to be natural. However, the standard regression of y and x has been universally used by comparative physiologists to the present. In the "Results" section, regression lines are calculated using both forms of regression analysis, but to prevent confusion, the relationships discussed in the final section are all derived with standard predictive regression analysis.

Results

The relationships of length (L), wet weight (W_w), and dry weight (W_d) to external surface area are presented in Fig. 2–4. In Fig. 5, oxygen consumption (R) at two experimental temperatures is plotted against wet weight. These relations are best fitted by power functions (Table 1) and the geometric mean regression gives the least biased estimate of the regression coefficient (Ricker 1973). To compare the present results with earlier work, standard regression coefficients are also given in Table 1.

In Fig. 3 the surface area of an oblate spheroid, 8 times as long as wide, is plotted against its calculated wet weight assuming a density of 1.0 g/cm^3. As expected, this underestimates the surface area of a euphausiid because any simple geometric approximation to body shape totally neglects the surface area of the appendages and "gills."

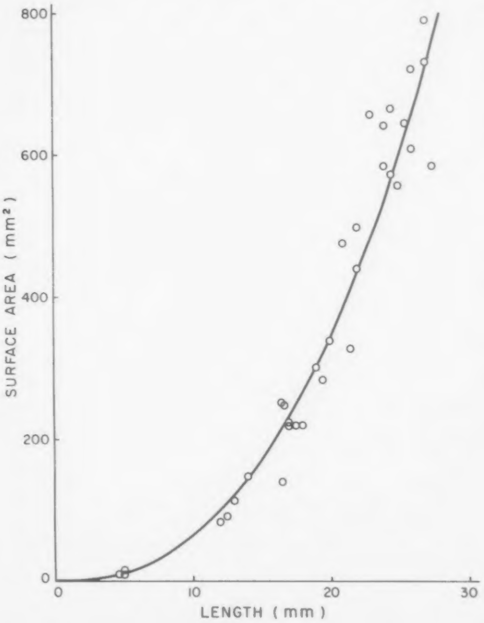

FIG. 2. Relationship between surface area and body length of *T. raschii*. $S = 0.24\ L^{2.4}$, $r = 0.98$.

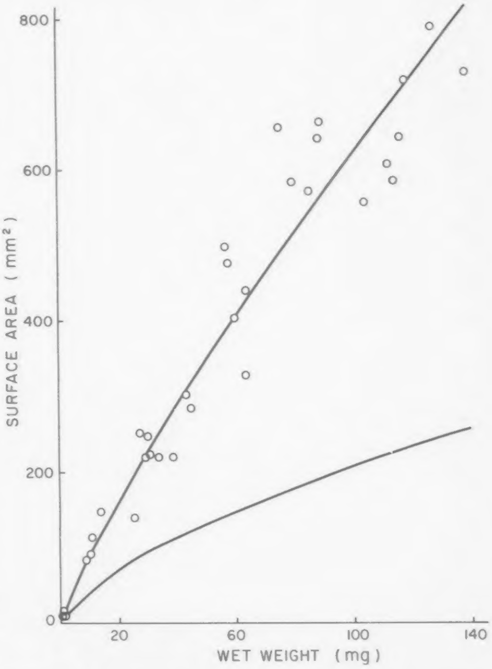

FIG. 3. Relationship between surface area and wet weight of *T. raschii*. $S = 13.0\ W^{0.84}$, $r = 0.98$. Lower curve, hypothetical surface area of the euphausiid form approximated by an oblate spheroid with a density of 1. $S = 10\ W^{0.66}$.

Discussion

The surface area of an organism with a fixed form is proportional to the square of any linear dimension, such as body length (L^2). Similarly, the volume will be proportional to the cube (L^3). If the density does not vary with the size of the organism, the weight (W) must be proportional to the cube of body length. It follows that the surface area (S) is proportional to the weight to the ⅔ power,

$$S = aW^{2/3}, \tag{1}$$

where a is the proportionality coefficient determined by the shape and dimensions of the organism. If respiration rate as a measure of metabolism, is proportional to surface area then

$$R = cW^{2/3} \tag{2}$$

and

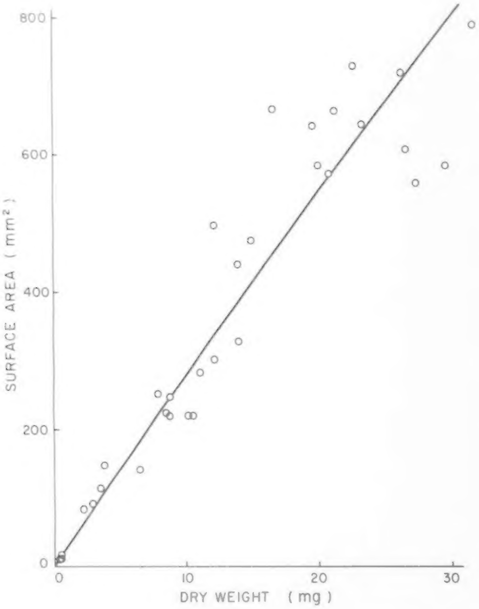

FIG. 4. Relationship between surface area and dry weight of *T. raschii*. $S = 33.1\ W^{0.95}$, $r = 0.97$.

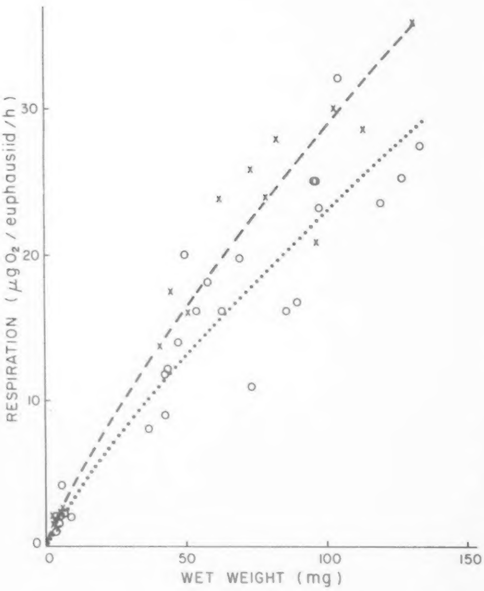

FIG. 5. Relationship between respiration rate and wet weight of *T. raschii* at 5.6°C (X) where $R = 0.67\ W^{0.82}$, $r = 0.98$ and at 1.2°C (○) where $R = 0.53\ W^{0.82}$, $r = 0.93$.

$$\frac{R}{W} = cW^{-\frac{1}{3}}$$

where c is the proportionality coefficient. This is the well known surface law which states that the metabolic rate per unit weight decreases with increasing size of organism but is constant per unit surface.

Surface area has proved to be a difficult variable to measure (Brody 1945); therefore, most investigators have used respiration rates together with body weights or lengths to imply either conformity or disagreement to the surface law (Bertalanffy and Krywienczyk 1953).

There are extensive compilations in the literature on the respiration–weight relationships for various animal groups. Brody (1945) concluded that a weight exponent value of 0.73 was typical of birds and mammals. Kleiber (1961) arrived at a figure of ¾ for mammals and Winberg (1956, 1961) gave 0.81 for freshwater fish and 0.79 for marine fish. Sushchenya (1972) recently collated the crustacean literature on respiration and size as follows:

Anostraca	$R = 0.08\ W^{0.70}$
Notostraca	$R = 0.45\ W^{0.81}$
Cladocera	$R = 0.14\ W^{0.80}$
Copepoda	$R = 0.2\ W^{0.77}$
Isopoda	$R = 0.14\ W^{0.82}$
Amphipoda	$R = 0.14\ W^{0.79}$
Caridea	$R = 0.23\ W^{0.87}$
Reptantia	$R = 0.09\ W^{0.84}$

Clearly, equations (1) and (2) should be rewritten in their general form,

$$S = aW^b \tag{3}$$

and

$$R = cW^d \tag{4}$$

Present results demonstrate that euphausiid respiration is proportional to $W_w^{0.82}$ at 5.6 and 1.2°C while the proportionality coefficient differs (Fig. 5). This concurs with Sushchenya's summary of the crustacean literature where the weight exponent is an innate property of the organism and the proportionality coefficient is affected by environmental factors such as temperature. (Exceptions to this are reported by Champalbert and Gaudy [1972] and Conover [1960] who found the weight exponent was also affected by temperature.)

Small and Hebard (1967) found that respiration of *Euphausia pacifica* was proportional to $W_d^{0.968}$, $W_d^{1.002}$, and $W_d^{0.955}$ at 5, 10, and 15°C, respectively. The exponent in each case was not

TABLE 1. Equations fitted by linear regression analysis after double logarithmic transformation. L = length (millimetres); S = surface area (square millimetres); W_w = wet weight (milligrams); W_d = dry weight (milligrams); R = oxygen consumption (micrograms of oxygen per individual per hour).

Variates		Standard regression analysis			Geometric mean regression analysis			
x	*y*	Fitted coefficient	Regression coefficient	Equation	*r*	Fitted coefficient	Regression coefficient	Equation
L	S	0.240	2.426	$S = 0.24\ L^{2.426}$	0.980	0.239	2.474	$S = 0.239\ L^{2.474}$
W_w	S	13.0	0.838	$S = 13\ W_w^{0.838}$	0.978	13.0	0.856	$S = 13\ W_w^{0.856}$
W_d	S	31.1	0.954	$S = 31.1\ W_d^{0.954}$	0.972	31.4	0.980	$S = 31.4\ W_d^{0.98}$
W_w	R (5.6 °C)	0.670	0.817	$R = 0.67\ W_w^{0.817}$	0.984	0.670	0.830	$R = 0.67\ W_w^{0.83}$
W_w	R (1.2 °C)	0.525	0.820	$R = 0.525\ W_w^{0.82}$	0.929	0.525	0.882	$R = 0.525W_w^{0.882}$

significantly different from 1. This was confirmed by Paranjape (1967) with the same organism at 5, 10, 15, and 20°C. This discrepancy with the present results might be due to the use of dry instead of wet weight. The majority of references in English cited by Sushchenya (1972) for Isopoda, Amphipoda, Caridea, and Reptantia used live (i.e. wet) weight. Individual Russian references could not be checked but Ivlev and Yakovleva (1963) do state that live weight is usually used. If dry weight is a constant percentage of wet weight, respiration would be proportional to wet and dry weight to the same exponent. However, measurement of *Thysanöessa raschii* shows,

$$W_d = 0.41\ W_w^{0.872},\ r = 0.99$$

where the exponent has a 95% confidence interval of ±0.029. This implies that larger euphausiids have a greater water content which cannot simply be explained as an artifact of differential desiccation during weighing. Gilchrist (1959) reported a similar wet weight–dry weight relationship with the brine shrimp, *Artemia salina*. With this interpretation the euphausiids no longer appear to be an exception to the crustacean equation,

$$R \propto W^{\sim 0.8} \text{ (Hemmingsen 1960)}$$

It follows from

$$R = cW_w^{0.82 \pm 0.05}$$

and

$$S = aW_w^{0.84 \pm 0.04}$$

(where both exponents have a 95% confidence interval) that respiration rate should be roughly proportional to surface area. That is when the exponents in equations (3) and (4) are equal,

$$R = \frac{c}{a} S \qquad (5)$$

Plots of respiration rate against surface area partially support this contention with $R = 0.034\ S^{1.01}$ at 1.2°C and $R = 0.024\ S^{1.09}$ at 5.6°C (Fig. 6). Analysis of covariance showed no difference between regression coefficients, $0.2 < P > 0.1$, but the coefficient for 5.6°C was significantly different from one. The fact that the euphausiids were living in 1–2°C water may help to explain the difference. Large individuals of a species may show a greater rate change with increased temperature than do small individuals (Bullock 1955; Rao and Bullock 1954) and this appears to be the case at 5.6°C in Fig. 6.

Few physiologists have quantified the respiratory surface area of animals other than mammals for comparison with their metabolic rates. Ellenby (1945) estimated the surface area of fruit fly puparium (*Drosophila*) by compressing the cast, following emergence, between two microscope slides, and measuring the combined area of the projected images of both sides with a planimeter. Oxygen uptake per unit surface area was found to be constant over the range of wet weight

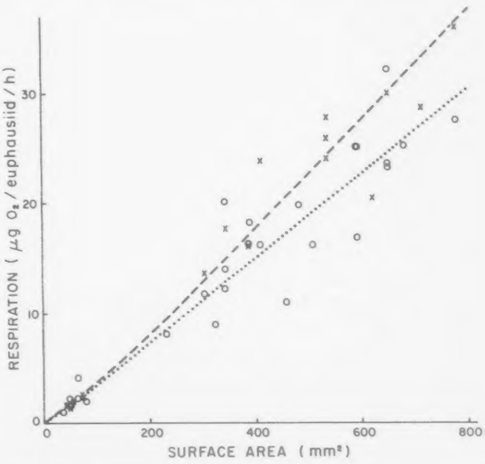

FIG. 6. Relationship between respiration rate and surface area of *T. raschii* at 5.6°C (X) where $R = 0.02\ S^{1.09}$, $r = 0.98$ and at 1.2°C (○) where $R = 0.03\ S^{1.01}$, $r = 0.94$.

used, i.e. respiration is proportional to surface area in *Drosophila* puparia. Anderson's (1970) data on six species of spiders, however, recalculated shows that

$$S_r \propto W_\omega^{0.84}$$

and

$$R \propto W_\omega^{0.63}$$

where the respiratory surface (S_r) of lung books was estimated from 10 to 20% of the serial cross sections of known thickness. Muir (1969) concludes that the geometric approximation of fish gill surface area is also a power function of wet weight, with the exponent ranging from 0.8 to 0.9, depending on the species. As previously stated, respiration rate is proportional to wet weight$^{0.81}$ in fishes (Winberg 1956).

Ultsch (1974) finds,

$$S_r \propto W_\omega^{0.65}$$

and

$$R \propto W_\omega^{0.66}$$

for submerged salamanders, calculating surface area both by fitting aluminum foil and geometric approximation. Tenney and Tenney (1970) present contrary evidence that the respiratory surface area of seven species of amphibians is proportional to $W_w^{0.98}$. Their explanation is that the respiratory surface they measured is not fully supplied with capillaries as is almost the case in mammals. They calculate that the "true" respiratory surface area varies as $W_w^{0.8}$. In the same paper, these authors report the respiratory surface area of 15 species of reptilians to be proportional to weight to the expected exponent of 0.75. Tenny and Remmers (1963) calculated the lung surface area for 28 species of mammals to show convincingly that,

$$R \propto S_r$$

from bats to whales. A linear relationship between respiratory surface area and metabolic rate appears to be the rule throughout the animal kingdom. It would be erroneous to conclude that the respiratory surface area limits the metabolic rate of an organism. Tenny and Remmers have pointed out that a porpoise has a much greater metabolic rate and alveolar surface area than a manatee of the same body weight. This must mean that both respiratory surface area and metabolic rate are adaptive characters which have evolved simultaneously with the organism.

The value and significance of the exponent in equations (3) and (4) has long been debated by researchers with experience on a particular organism or group of organisms, i.e. a small size range. Hemmingsen (1960) demonstrates in a comprehensive monograph on size–rate relationships that respiration rate is proportional to weight to a power of roughly ¾ for the smallest microorganisms through to whales. Viewed from this scale, the weight exponent of ¾ appears to be common to life, which suggests that its explanation is likely to be found in the functioning of the individual cell. Davison (1955, 1956) proposed a surface-area limitation of metabolism in metazoans but at the cellular level. Davison (1956), working with the crayfish *Procambarus*, demonstrated that respiration rate was proportional to $W_w^{0.76}$, and surface area of individual muscle fibers (assuming an open-ended cylindrical shape) was proportional to $W_w^{0.78}$ of the entire organism. Ellenby (1953), however, found no effect of cell size on oxygen uptake from observations on diploid and triploid (larger cells) prepupae of *Drosophila*. Smith (1956) reports that oxygen consumption of mammalian liver homogenate varies with $W_d^{0.69}$ and that the calculated total mitochondrial surface area of liver is proportional to $W_d^{0.72}$. It is conceivable that the weight exponent is determined by the transport of either respiratory gases or metabolites to and from subcellular surfaces.

The fact that respiration rate is proportional to respiratory surface area to the power of 1 does not enable us to predict the rate. Three groupings of metabolic rate are apparent from Hemmingsen's large scale of presentation. Homeotherms have a metabolic rate about 28 times that of poikilotherms of similar size and poikilotherms are about 8 times more metabolically active than unicellular organisms per unit weight. These differences in rates are represented by the coefficient *c* in equations (4) and (5) which up to now has been noted to reflect environmental parameters such as temperature. The value of *c* is then also determined by the physiological adaptations of the organism. The residence time of air or water on the respiratory surface, the permeability of the respiratory surface, and the efficiency of the circulatory system in collecting and distributing oxygen to the body cells have all been optimized to a different degree in unicellular organisms, poikilotherms, and homeotherms.

In conclusion, the surface to volume relationship which exists for a three-dimensional shape of constant proportions cannot be extended to encompass most organisms. The failure of euphausiids to comply to the relationship $R \propto W^{\frac{2}{3}}$ is therefore not because respiration rate is not proportional to surface area but because euphausiids do not retain a constant shape ($S \neq aW^{\frac{2}{3}}$) or density throughout life. Indeed, respiration rate is proportional to respiratory surface area in most organisms studied. Metabolism is universally related to some power of weight near ¾, from bacteria to whales, which suggests that this is a property of the cell. (The value of ¾ is certainly low in the light of Ricker's [1973] recent paper on regression analysis.) The metabolic rate is clearly the result of phylogeny and environmental factors.

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Changes in the Size Structure of Cichlid Populations of Lake Malaŵi Resulting from Bottom Trawling

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TURNER, J. L. 1977. Changes in the size structure of cichlid populations of Lake Malaŵi resulting from bottom trawling. J. Fish. Res. Board Can. 34: 232–238.

A bottom trawl fishery, primarily for cichlids, was started in 1968 in southern Lake Malaŵi. Since then a dramatic change has occurred in the catch of cichlids from one dominated by large species to one dominated by small species. Rates of change in abundance and mean length were much greater in the southern end of the southeast arm (area A) which has been fished for a longer period using trawls with 25-mm codend mesh than in the central region of the southeast arm (area B) which has been fished for a shorter time using trawls with 38-mm codend mesh. The changes in area A suggest heavy fishing mortality with little recruitment for species over 200 mm maximum length, heavy fishing mortality with limited recruitment for the medium-sized species (180–200 mm maximum length), and light fishing mortality and excellent recruitment for the small species (140–180 maximum length). Changes in area B showed the same trends but were considerably less drastic. The number of species caught has declined over 20% in the heavily fished areas. The remaining common species have greatly expanded their distribution. The change in the relative abundance of large and small species in the catch, and the decline in the number of species, appear to be stabilizing. These changes are probably the result of the size-selective nature of the codend mesh of the trawl net resulting in excessive fishing mortality for the larger species.

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Une pêche au chalut de fond, surtout de cichlidés, débuta en 1968 dans le lac Malawi méridional. Il s'est produit depuis un changement dramatique dans les prises de cichlidés : les grandes espèces qui dominaient au début ont été remplacées par les petites espèces. Les taux de changement d'abondance et de longueur moyenne ont été beaucoup plus prononcés dans l'extrémité méridionale du bras sud-est (région A) qui avait été exploitée sur une plus longue période à l'aide de chaluts de mailles de poche de 25 mm que dans la région centrale du bras sud-est (région B) qui avait été pêchée sur une plus courte période à l'aide de chaluts de mailles de poche de 38 mm. Les changements qui se sont produits dans la région A suggèrent une forte mortalité due à la pêche avec faible recrutement pour les espèces de longueur maximale de plus de 200 mm, une forte mortalité due à la pêche avec recrutement limité pour les espèces de taille moyenne (180–200 mm de longueur maximale) et enfin faible mortalité due à la pêche et excellent recrutement pour les petites espèces (140–180 mm de longueur maximale). Les changements qui se sont produits dans la région B démontrent les mêmes tendances, mais ils sont beaucoup moins dramatiques. Le nombre d'espèces capturées a diminué de plus de 20% dans les régions fortement pêchées. Les espèces communes qui restèrent élargirent considérablement leur aire de distribution. Le changement dans l'abondance relative des grandes et des petites espèces dans les prises, de même que le déclin du nombre d'espèces semblent se stabiliser. Ces changements résultent probablement de la sélection des tailles par les mailles de la poche du chalut entraînant une mortalité par pêche excessive des grandes espèces.

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LAKE Malâwi has a unique fish fauna with more known species than any other lake in the world. Of the 250 described species, more than 200 belong to the family Cichlidae, and of these, all but four are endemic. Experimental bottom trawling in 1965 revealed the presence of a commercially exploitable stock consisting mainly of small cichlids, and in 1975, 7000 t of fish were caught by the nine trawling units engaged in the fishery. A great deal of concern has been expressed over the possible effects of commercial trawling on the

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fish population of Lake Malâwi and the other Great African Lakes (Fryer 1972a, 1972b, 1973, Fryer and Iles 1972; Stoneman et al. 1973; Jackson 1973). Turner (1977a) has demonstrated a dramatic change in the species composition of the catch in the heavily exploited southern end of Lake Malâwi from one dominated by large species of cichlids to one dominated by small species. This paper analyses these changes in detail.

Materials and Methods

Exploratory trawl surveys were conducted with the 88 hp vessel, *Ethelywnn Trewavas*. An otter trawl with a 17-m headrope, 20-m footrope, and 25-mm stretched mesh codend was used throughout the survey. Each tow was made for 30 min at a speed of approximately 3.7 km/h (2 knots). To date, a significant amount of commercial fishing has taken place only in areas A and B (Fig. 1). Commercial trawling started in area A in 1968 using only a 25-mm codend mesh and in area B in 1972 using only a 38-mm codend mesh. Exploratory surveys were conducted in these areas in March and July 1971; March, July, and October 1972; January 1973; June and December 1974; and June 1975. Three stations were sampled in area A and four in area B. At each station two to four tows were made parallel to the shore at depth intervals of approxiamtely 20 m. An echo sounder recorded the exact depth of each tow.

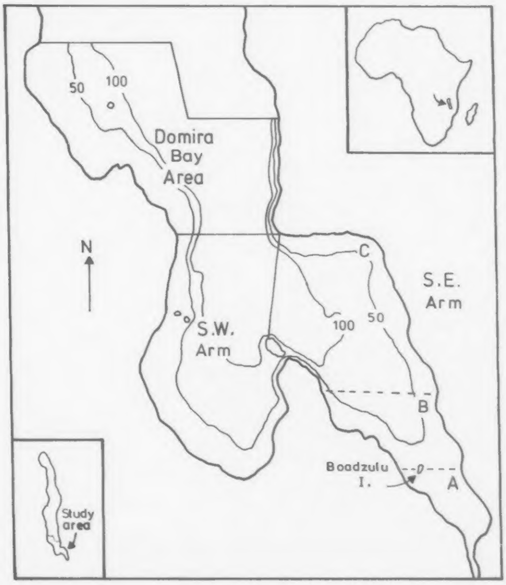

FIG. 1. Sampling areas at southern end of Lake Malâwi. Depth contours in meters.

The catch was sorted into four categories: clariid catfish, *Bagrus meridionalis*, "small cichlids" primarily *Haplochromis* and *Lethrinops* species, and "others" (including mormyrids, cyprinids, and large cichlids such as *Tilapia*). Only the category "small cichlids" was used in this analysis. A sample of approximately 20 kg was collected by taking shovelfuls of fish from different parts of the catch while on deck. Samples were stored in Formalin.

Th taxonomy of Lake Malâwi cichlids is very difficult as more than 160 closely related species are taken in trawl catches. Specimens in the sample were identified to species and each weighed individually to obtain the percentage composition of the catch. These sample values were multiplied by the weight of the total catch to obtain an individual species catch per effort. Only the more common species (Table 1), i.e. those with an average catch rate >10 kg/h for any one sampling period, were used for the analysis of change in abundance. Total lengths of individuals of the common species were measured to the nearest millimetre. Only those species with sample size of at least 100 individuals were used for assessing changes in mean length.

The change in abundance of each species was determined by fitting a linear trend line by the method of least squares to the catch per effort for the nine sampling periods from March 1971 to June 1975. The change, determined in kilograms per month was then compared with the logarithm of maximum length for each species to render the results linear. The maximum length of each individual species is defined as the length of the largest specimen caught.

Results

CHANGE IN ABUNDANCE

The relationship between change in abundance of individual species and maximum length is highly significant in area A (Fig. 2); species with

TABLE 1. Species used in the analysis.

	Area A	Area B
Haplochromis anaphyrmus Axelrod	x	x
H. argyrosoma Regan	x	x
H. atritaeniatus Regan	x	x
H. intermedius (Gunther)	x	
H. longimanus Trewavas	x	
H. mloto Iles	x	x
H. sphaerodon Regan	x	x
Lethrinops alta Trewavas		x
L. aurita (Regan)	x	x
L. longimanus Trewavas	x	x
L. longipinnis Eccles & Lewis	x	x
L. macracanthus Trewavas	x	x
L. parvidens Trewavas	x	x
L. stridei Eccles and Lewis	x	
Pseudotropheus elegans Trewavas	x	x
P. livingstonii (Boulenger)	x	x

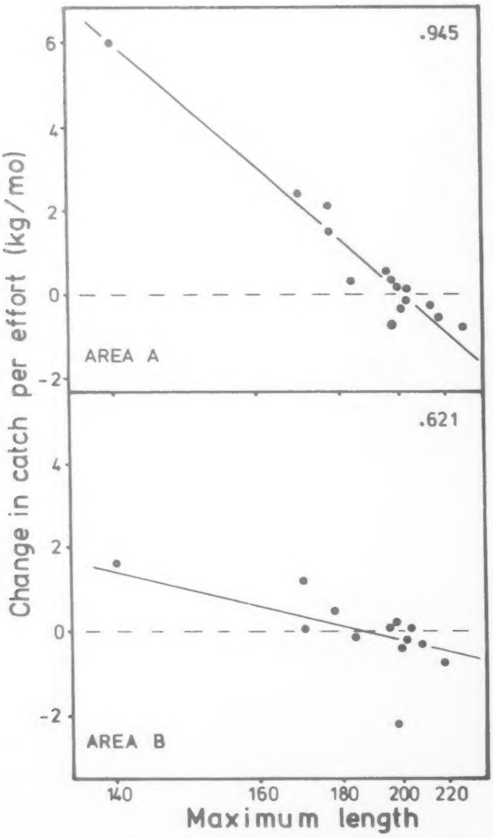

FIG. 2. Change in catch per effort from March 1971 to June 1975 and maximum length in areas A and B. Points on figure represent individual species. Correlation coefficient is in upper right corner.

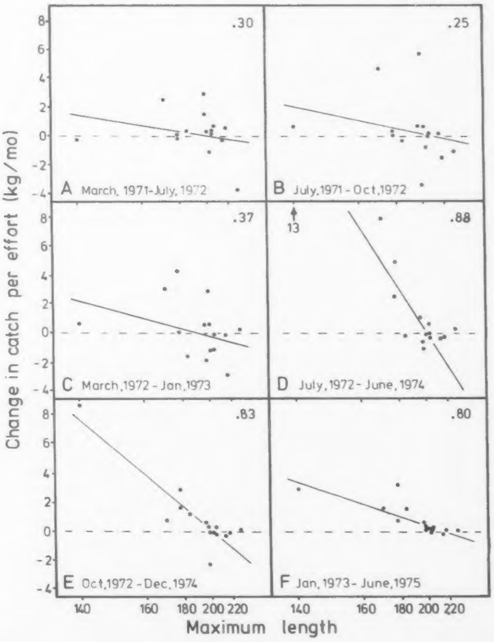

FIG. 3. Change in catch per effort for various time periods and maximum length in area A. Correlation coefficient is in upper right corner.

a maximum length <200 mm increased. The relationship is also significant in area B, and if *L. stridei*, the lowest point in the figure, is not considered, it is highly significant ($R = 0.81$).

An attempt was made to monitor these changes over time by calculating the rate of change in catch per effort for the first four sampling periods, the second to fifth period, third to sixth period, and so on until all nine surveys were covered. Note that the individual time periods vary in length and are not strictly comparable. The relationship between change in abundance and maximum length was not significant in area A until the July 1972 to June 1974 period (Fig. 3D) but has remained highly significant since. A negative slope of change is evident throughout all time periods. The greatest rate of change occurred during the July 1972 to June 1974 period and was largely due to the great increase in abundance of the four species <180 mm. The rate of change has decreased rapidly in the last two time periods (Fig. 3E and F).

A similar comparison between change in abundance and maximum length over time was made in area B. The same procedures of analysis were used as in area A except that, for reasons given later, *L. stridei* was not considered in the calculation. The relationship between change in abundance and size became significant during the last two periods (Fig. 4C and F). A negative slope of change was evident in every period, except March 1972 to January 1973 (Fig. 4C). The greatest rate of change occurred during October 1972 to December 1974 (Fig. 4E). The rate of change in area B was always less than for area A.

As a control, a comparison of change in abundance with maximum size was made for the unfished southwest arm. There was no change in abundance by maximum size in this area.

CHANGE IN MEAN LENGTH

A comparison of the change in average length of individual species from 1971 to 1973 in area A

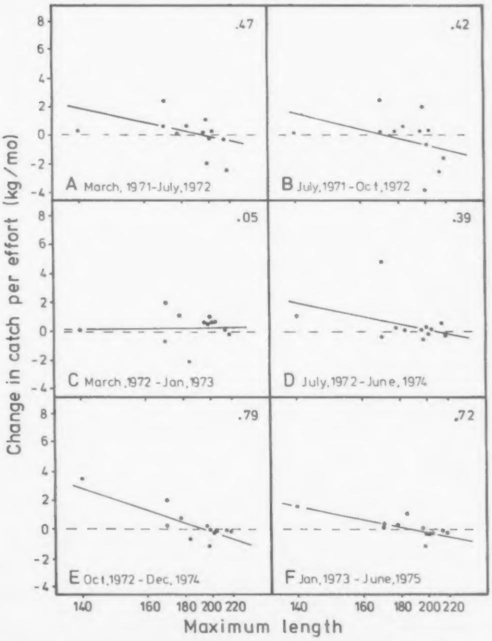

FIG. 4. Change in catch per effort for various time periods and maximum length in area B. Correlation coefficient is in upper right corner.

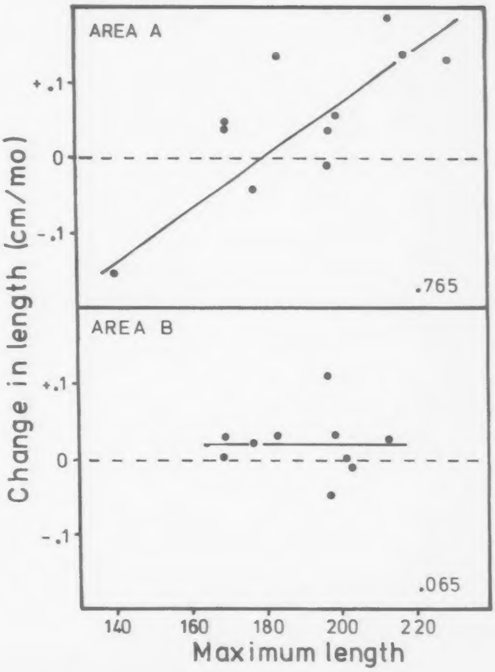

FIG. 5. Change in mean length from 1971 to 1973 and maximum length. Correlation coefficient is in lower right corner.

with the maximum length of each species shows a highly significant relationship, the greater the maximum length the greater the rate of increase in mean length (Fig. 5). Similar comparison in area B (Fig. 5) and in the nonfished southwest arm showed no such correlation. Assuming a constant rate of recruitment and growth, the mean length of individual species would be expected to decline with heavy fishing mortality. Just the opposite occurred with an increase in mean length of 8 of 11 species, particularly for those larger than 180 mm. Limited or no recruitment for the larger species appears to be the most probable cause for this observation although an increase in growth rate in response to reduced population density may be partially responsible. Comparable length data from the exploratory catch were not available for 1974.

ABUNDANCE–MEAN LENGTH TRAJECTORY

The abundance (catch per effort data) of a species and its mean length in the catch are affected differently by changes in mortality, growth, and recruitment. Their combined effects over time should provide more information on the source of changes than a consideration of each independently (Henderson 1972). The average abundance and mean length of several species were grouped by size to study the abundance–mean length trajectory as related to maximum length. Considerable differences are evident between the different size-groupings in area A (Fig. 6). The larger species (over 200 mm maximum length) experienced a rapid decrease in abundance and increase in mean length from 1971 to 1973 suggesting heavy fishing mortality with little or no recruitment. A decrease in mean length by 1975 suggested limited recruitment at a very low level. The medium-sized species (180–200 mm) decreased in abundance and increased in mean length but not to the extent of the larger size-group. An increase in abundance was evident after 1973 with a decrease in mean length. The small size-group (140–180) rapidly increased in abundance from 1971 to 1973 but did not decrease in mean length until 1975.

Much less drastic changes occurred in area B (Fig. 6). The larger species fluctuated in abundance and generally decreased in mean length. The medium and small species increased in abundance but decreased in average length.

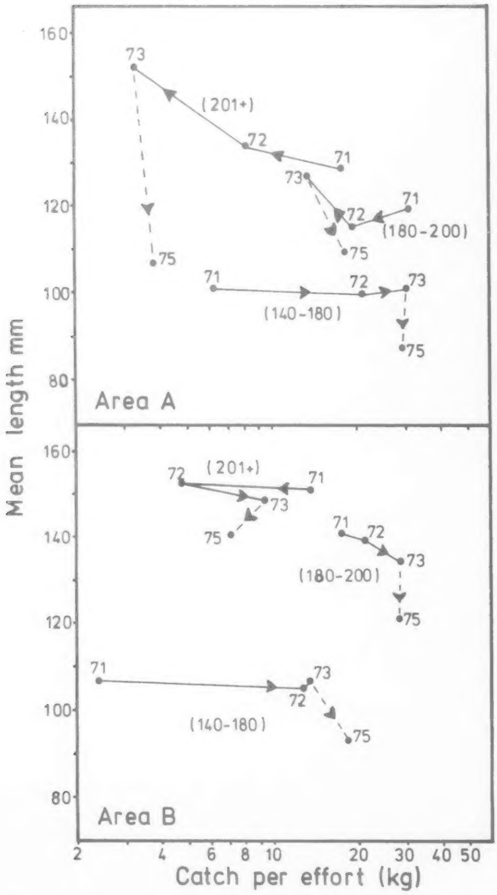

FIG. 6. Mean length–abundance trajectory over time for various size-groups of cichlids in areas A and B. Numbers on figure designate years. Comparative mean length data not available for 1974.

Heavy fishing mortality and the size selective nature of the codend mesh of the trawl net appear to be responsible for these changes. Most of these cichlids were initially caught at approximately the same length (Turner 1977b). However, large species which grow to a larger maximum length are caught at a much younger age and, therefore, are subjected to greater fishing mortality than small species. There is probably a close stock–recruitment relationship for cichlids as they produce few eggs and depend heavily on parental care for survival of young. As a result, reduction of the brood stock of larger species resulted in few eggs and larvae and very limited recruitment to the fishery. The decline in abundance of the large-, and to a lesser extent the medium-sized species, created a larger food supply and less predation on the smaller species. This resulted in improved growth and survival rates with a rapid increase in the abundance of small species.

CHANGES IN SPECIES DIVERSITY

A species count is the simplest measure of the species diversity or richness of an area but is dependent on sample size. This can be solved by plotting the cumulative number of species against the cumulative number of samples. This was done for the time periods 1971–72, 1972–73, 1973–74, and 1974–75 (Fig. 7A) to measure the effects of trawling on species richness over time in areas A and B. These data were summed over a 2-yr period to obtain an adequate sample size. A rapid decline in the number of species occurred in the heavily fished area from 1971–72 to 1973–74 with the decline stabilizing by 1974–75. As a

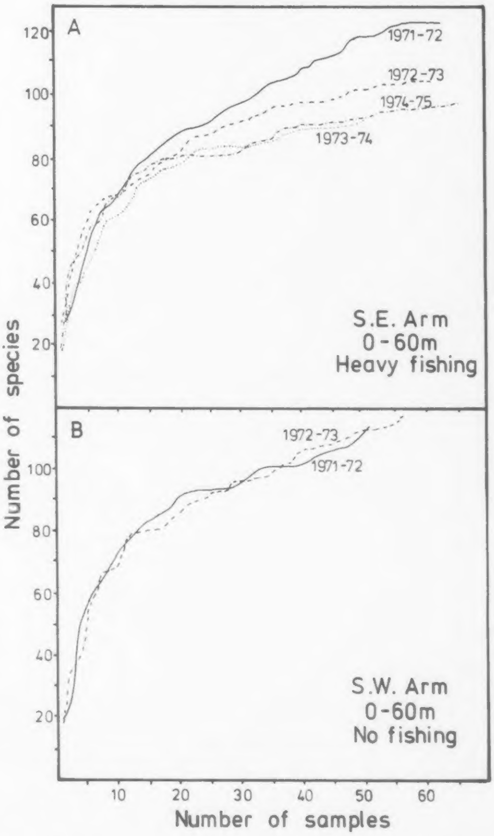

FIG. 7. Accumulative number of species taken in accumulative number of samples over time.

control, a comparison of species richness with time was made in the nonfished southwest arm (Fig. 7B). No decline in species richness was evident.

Change in Species Distribution

Changes in species distribution with time were examined by calculating the average percent occurrence of the more common species in the catch for each sampling survey. A change in distribution would be reflected in the number of times a species occurred at the various sampling stations. Thirty-two species were defined as common, having occurred in at least 50% of the samples in any one survey. The relationship between occurrence and time was highly significant ($R = 0.95$) with a doubling of percent occurrence over a 5-yr period (Fig. 8).

A factor that may bias this relationship is that the number of specimens examined per sample may have increased over time. The sample size (20 kg) remained the same throughout the study but the length of fish decreased with the result that more individuals are examined per sample. The increase in the number of fish examined with time is unknown, but in a recent test of six samples, a doubling of sample size from 20 to 40 kg resulted in an 8% increase of occurrence, which is certainly not sufficient to compensate for the doubling in percent occurrence observed.

Several authors have demonstrated that the distribution of closely related species is restricted when they live sympatrically, which suggests an interspecific competition effect. As a result of the trawl fishery there has been a major reduction in the number of species and, therefore, a theoretical reduction in interspecific competition. In addition, populations of *B. meridionalis* and clariid catfish, major predators in Lake Malâwi, have declined over this period (Turner 1977a). The combination of a reduction in predation by catfish species and less interspecific competition probably resulted in the greatly expanded distribution of the remaining cichlids.

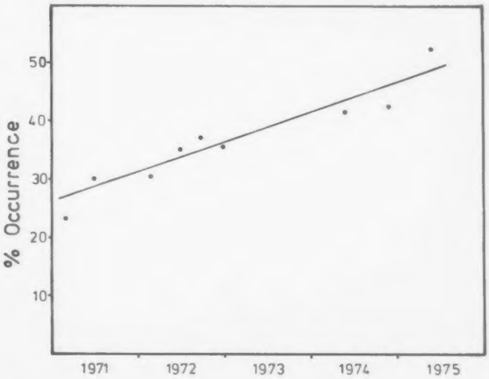

FIG. 8. Average percent occurrence of the 32 most common species of cichlids caught in trawl hauls from 1971 to 1975.

Discussion

Little is known about the movements of the demersal fish stocks in Lake Malâwi, yet our estimates of yield assume that a rather discreet population exists in each area (Turner 1977a). The large increase in the catch rate in area A in 1974 certainly suggests a large movement of fish into the area from the north. Commercial fishing did not start in area B until November 1972, yet a decrease in abundance of some of the larger species occurred before fishing started (Fig. 4A), suggesting some movement of species between area A and B. Surprisingly, there was no evidence of a change in mean length from 1971 to 1973 in area B (Fig. 5).

Lethrinops stridei was not used in the analysis of area B as it rapidly declined in abundance even before fishing started in that area. This was the only commercially important species that was not common in all of the areas sampled (Turner 1977a). Large catches were taken in the exploratory trawl catch only at the sampling stations near Boadzulu Island, the dividing line for area A and B. This is also the portion of area B nearest to the fish landing sites in area A. This species must have moved freely between the two areas before fishing started in area B, and was subjected to high fishing mortality in area A.

The change in species composition from large to small species and the loss of a number of species do not appear to have affected either the catch per effort or the size of commercial trawl catch. Turner (1977a) found evidence of overfishing in area A in 1971 and 1972; however, a reduction in trawling effort in 1973 and 1974 resulted in increased catch rates equal to that for 1968–70 when the fishery first started. The 1973 and 1974 catches were obviously composed of fewer, smaller, species but the total yield was similar. The market for fresh fish has seldom been saturated and most fish are consumed locally regardless of size or species.

The greatest rate of change from large to small species in area A occurred from July 1972 to June 1974, largely due to the increase in abundance of species <180 mm. The excessive fishing mortality in 1971 and 1972 probably greatly accelerated these changes. The rapid increase in small species following the decline of the large species suggests considerable interspecific competition. Further excessive fishing mortality could accelerate a shift to still smaller species resulting in reduced yield if a large percentage of the biomass were present as smaller species — too small to be taken effectively by the present 25-mm codend. Approximately 20% of the species in Lake Malâwi have a maximum recorded length of 140 mm or less (Fryer and Iles 1972).

A number of fisheries have progressed from large to smaller mesh sizes as the species composition changed from large to small species. Christie (1968) and Regier and Loftus (1972) pointed out that when a fishery shifts its efforts to a smaller species after the decline of a stock of a larger species, and if a smaller mesh is used for the new stock, the capture of juveniles of the larger species may in effect permanently suppress that species. Size selectivity of fishing gear and/or eutrophication has greatly changed the deepwater cisco population (*Coregonus*) in Lake Michigan (Smith 1968). Of the original seven species that inhabited the lake, the two largest have become extinct, the four species of intermediate size have seriously declined, and the smallest has become very abundant.

The question of what size of codend mesh to use presents a difficult problem. Any mesh size that will catch a wide range of species will give a definite competitive edge to the smaller species resulting in the decline of larger species. A very small mesh net would catch all species equally well but would catch juveniles of all species.

Recommendations have been made to the Malâwi Government to limit the amount of effort in area A to the potential yield based on catch–effort data (Turner 1977a). Recommendations were initially made for the adoption of a uniform codend mesh of 38 mm in anticipation that this would allow more individuals of the large species to reach maturity in area A. Now, however, a change from a 25- to 38-mm codend would result in a substantial reduction in catch in area A. In addition, it is doubtful if they would recover to their former abundance as a 38-mm codend has always been used in area B where the larger species have still declined.

The change from large to small species and the decline in the number of species appear to be stabilizing in the present heavily trawled areas of the lake. The fishery is expanding to other regions of the lake where similar changes are to be expected. Adoption of a 38-mm codend, and probably more important, limiting the fishing effort in the remainder of the lake should help to minimize the effect of these changes.

Jackson (1973) states the African lakes should be managed to provide the maximum yield of protein for ever-increasing populations while conserving the world's richest lacustrine fish fauna. This study suggests that it may be impossible to do both and that to maximize the yield of protein there will be a change in species composition and a decline in the number of endemic species in trawled areas. This does not necessarily mean that these species have been locally extinguished, though they may have been. The common species are rather evenly distributed among both the fished and unfished areas studied (Turner 1977a) suggesting that most species are protected against local depletion. However, rare species are apt to have local distributions. Further studies are certainly needed on the biology and population dynamics of this unique and valuable resource.

Acknowledgments

This paper was written as part of the stock assessment program of FAO/UNDP/Malâwi Government project for the promotion of integrated fishery development. The views expressed are mine, not necessarily those of the above-mentioned organizations. I thank D. Eccles for supervision of the species identification, H. Klokk, J. Tarbit, D. Tweddle, the crew of the *Ethelwynn Trewavas*, and all the laboratory workers at Monkey Bay Research Laboratory especially L. M. Phiri.

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Preferred, Avoided, and Lethal Temperatures of Fish During Rising Temperature Conditions

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Selected temperatures increased for 15 fish species in a laboratory gradient when acclimated to 3°C increments from 12 to 27°C. Temperature preferences exceeded acclimation temperatures between 12 to 27°C for all except the telescope shiner (*Notropis telescopus*), yellow perch (*Perca flavescens*), rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*). At 30, 33, or 36°C acclimation, preferred temperatures were less than the acclimation temperature. The highest temperature preferences occurred at acclimations of 27, 30, or 33°C. Bluegill (*Lepomis macrochirus*), spotted bass (*Micropterus punctulatus*), rockbass (*Ambloplites rupestris*), and the spotfin shiner (*Notropis spilopterus*) consistently selected the highest temperatures while the lowest temperatures were preferred by the salmonids. Final temperature preferences were usually greater than 29°C for most centrarchids, above 24°C for most cyprinids, and less than 19°C for salmonids.

Avoidance temperatures increased as acclimation temperature increased. Upper avoidances tested at high acclimation temperatures (30–36°C) either equalled or exceeded the 7-day upper lethal temperature limit of the species by 1–2°C, while avoidances were below this upper lethal limit when tested at the acclimation temperature closest to the species' final temperature preference.

CHERRY, D. S., K. L. DICKSON, J. CAIRNS JR., AND J. R. STAUFFER. 1977. Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. J. Fish. Res. Board Can. 34: 239–246.

Les températures choisies augmentent pour 15 espèces de poissons dans un gradient en laboratoire lorsque acclimatés à des augmentations de 3°C entre 12 et 27°C. Les températures préférées dépassent les températures d'acclimatation entre 12 et 27°C chez toutes les espèces, sauf le méné télescope (*Notropis telescopus*), la perchaude (*Perca flavescens*), la truite arc-en-ciel (*Salmo gairdneri*), la truite brune (*Salmo trutta*) et l'omble de fontaine (*Salvelinus fontinalis*). A des températures d'acclimatation de 30, 33 ou 36°C, les températures préférées sont inférieures à la température d'acclimatation. Les températures préférées les plus élevées se rencontrent à des températures d'acclimatation de 27, 30 ou 33°C. Le crapet arlequin (*Lepomis macrochirus*), l'achigan tacheté (*Micropterus punctulatus*), le crapet de roche (*Ambloplites rupestris*) et le méné bleu (*Notropis spilopterus*) choisissent invariablement les plus hautes températures, alors que les salmonidés préfèrent les plus basses températures. Les préférences finales de température sont ordinairement supérieures à 29°C pour la plupart des centrarchidés, supérieures à 24°C pour la plupart des cyprinidés et inférieures à 19°C pour les salmonidés.

Les températures d'évitement augmentent à mesure que la température d'acclimatation augmente. Les températures d'évitement supérieures essayées à de hautes températures d'acclimatation (30–36°C) sont égales à la limite supérieure de température létale après 7 jours pour l'espèce ou les dépassent de 1–2°C, alors que les températures d'évitement sont inférieures à cette limite létale supérieure lorsque essayées à la température d'acclimatation la plus rapprochée de la préférence de température finale de l'espèce.

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THE preferred, avoided, and lethal temperatures were determined for 15 fish species at a field laboratory during rising temperature conditions. The laboratory is located near the confluence of the thermally influenced New and East rivers, Glen Lyn, Virginia. The impact of thermal loading has prompted many investigations and literature reviews about this discharge, but few studies have analyzed the relationship of these temperature criteria at the power plant site during a full year of falling and rising temperature conditions. Specific advantages of this study over traditional laboratory investigations include the ease of evaluating fish responses to thermal discharges by reducing transportation time after capture, and in utilizing river water to acclimate and test fish responses (Cherry et al. 1975).

A temperature preference and avoidance review has been compiled by Coutant (1975), and differences between temperatures selected during rising versus falling conditions have been reported (Meldrim and Gift 1971; Sullivan and Fisher 1953). Temperature responses under various conditions and thermal histories are necessary for understanding and predicting fish movements in thermal discharges. In this study the responses between each temperature condition are analyzed and the final temperature preferendum for each species calculated. At acclimation temperatures closest to the final preferendum, the avoidance response is compared to the lethal temperature in order to provide information related to the optimum temperature range.

Materials and Methods

SOURCES OF FISH

Most fish were collected by seining in the New River drainage system and were immediately brought to the field laboratory and maintained there. Holding conditions, feeding, and acclimation procedures were reported in Cherry et al. (1975) where a description of the research site is also given. Five species, bluegill (*Lepomis macrochirus*), rainbow trout (*Salmo gairdneri*), brown trout (*S. trutta*), brook trout (*Salvelinus fontinalis*), and yellow perch (*Perca flavescens*) were obtained from state or commercial fish hatcheries since they were either uncommon or absent from the Glen Lyn site. All species except the cyprinids were 1 yr old or less, and no gravid individuals were used. Most species tested ranged from 50 to 100 mm fork length (FL) except yellow perch which were 30–50 mm FL.

TEMPERATURE PREFERENCE TRIALS

Group preference temperatures were obtained as described by Cherry et al. (1975). Data points were evaluated by a least squares regression analysis (with 95% confidence limits) between acclimation and preference temperatures (Sokal and Rohlf 1969). Statistical linear and curvilinear hypotheses were used to test the equality of regression lines calculated for the same acclimation temperature during falling conditions (Cherry et al. 1975) with those calculated for rising conditions. Statistical significance ($P = 0.05$) was obtained from the equation:

$$F = \frac{(SS_{e3} - [SS_{e1} + SS_{e2}])/\times}{SS_{e1} + SS_{e2}/df_1 + df_2}$$

where SS_{e1} and SS_{e2} are the sum of squares for the regressions during rising and falling temperature, SS_{e3} is the sum of squares of the joint regression (rising and falling conditions combined), df_1 and df_2 are the degrees of freedom for rising and falling temperatures, and × is the number of factors regressed in the regressions of rising and falling conditions minus the number regressed in the joint regression (Myers, Department of Statistics, Virginia Polytechnic Institute and State University, personal communication).

A stepwise regression procedure was used to analyze whether a linear or first to fifth order curvilinear regression could significantly ($P = 0.05$) fit the temperature preferences through the acclimation temperatures tested (Sokal and Rohlf 1969). From the best fit calculated, the final temperature preferences or temperature preferenda were obtained as the point where the regression line intersected a theoretical acclimation line with a slope of one (Fry 1947).

AVOIDANCE TRIALS

The avoidance trials were conducted in a trough described by Cherry et al. (1975) with some modifications in the gradient formation and in the statistical analysis. The trough consisted of two distinct temperature regions (T_1 and T_2), one on each side of the center drains. A control trial run consisted of T_1 and T_2 being equal to the acclimation temperature and after a 40-min orientation period (to as much as 120 min for slowly adjusting species) fish were continuously monitored for 10 min to establish a control or reference response. Responsive fish spent approximately 5 min on each half of the trough through continuous random movement. In successive trials, each with a 10-min monitoring period, first one side (T_2) was elevated by 3°C followed by raising both sides by 3°C with the higher side always being 3°C greater. This procedure attempted to simulate fish movement up the thermal gradient in the New River where temperature tended to increase upstream toward the discharge condensor pipes. Since statistically significant avoidances always occurred at the first 3°C increment for tests conducted at the highest acclimation temperature (e.g. $T_1 = 36$°C and $T_2 = 39$°C), adjustments were made in the gradient to identify the first specific temperature within this 3°C increment in which 9 min of the 10-min monitoring period was spent away from a temperature above acclimation ("absolute" avoidance). In this procedure, the center and side drains were manipulated to allow mixing to occur on each side of the drain which

TABLE 1. Preferred temperatures with (in parentheses) 95% confidence limits of 15 fish species, each tested in groups of eight with one replication, sampled continuously during rising temperature conditions from the New and East rivers Virginia, and from state fish hatcheries at acclimation temperatures from 12 °C in March to 36 °C in October.

	Acclimation temp (°C)								
	12	15	18	21	24	27	30	33	36
Cyprinidae									
Campstoma anomalum (Stoneroller)	16.5 (18.2–14.2)	17.0 (19.8–16.7)	21.0 (21.6–19.2)	22.4 (23.6–21.4)	25.1 (25.9–23.4)	28.2 (28.3–25.2)	27.4 (30.8–26.9)	—[b]	—[b]
Pimephales promelas (Fathead minnow)	19.5 (20.7–17.0)	21.2 (21.9–18.9)	20.9 (23.2–20.8)	22.0 (24.6–22.6)	25.4 (26.4–24.0)	27.6 (28.3–25.3)	28.7 (30.3–26.5)	—[b]	—[b]
Pimephales notatus (Bluntnose minnow)	19.3 (20.0–18.0)	20.9 (21.5–19.9)	21.9 (23.0–21.7)	23.2 (24.6–23.5)	26.4 (26.4–25.2)	27.9 (28.8–26.7)	29.0 (30.2–28.2	—[b]	—[b]
Notropis rubellus (Rosyface shiner)	20.8 (22.2–18.7)	21.7 (23.0–20.2)	22.2 (24.0–21.7)	22.5 (25.0–23.0)	25.8 (26.2–24.2)	28.1 (27.5–25.2)	28.0 (29.0–26.2)	27.7 (30.5–27.0)	—[b]
Notropis spilopterus (Spotfin shiner)	21.4 (24.4–19.3)	21.8 (25.2–21.0)	24.1 (26.2–22.7)	26.4 (27.2–24.3)	27.3 (28.4–25.7)	30.6 (29.8–26.9)	31.8 (31.4–28.0)	31.0 (33.1–28.9)	29.2 (34.8–29.8)
Notropis telescopus (Telescope shiner)	14.2 (16.3–11.5)	15.4 (18.0–14.4)	17.7 (19.9–17.0)	22.6 (22.1–19.3)	23.2 (24.8–21.2)	24.4 (27.6–22.8)	—[b]	—[b]	—[b]
Centrarchidae									
Lepomis macrochirus[a] (Bluegill)	24.1 (25.7–23.2)	25.2 (26.5–24.5)	26.8 (27.4–25.7)	27.8 (28.3–26.8)	28.2 (29.2–27.8)	30.0 (30.3–28.9)	32.4 (31.5–29.8)	30.9 (32.7–30.6)	31.8 (33.9–31.4)
Micropterus dolomieui (Smallmouth bass)	—[c]	20.2 (25.7–19.5)	25.5 (26.6–21.7)	25.8 (27.5–23.7)	28.2 (28.8–25.4)	29.7 (30.6–26.7)	30.9 (32.6–27.7)	29.4 (34.8–28.5)	—[b]
Micropterus punctulatus (Spotted bass)	—[c]	24.8 (24.4–20.5)	26.8 (28.2–25.6)	28.0 (28.8–26.8)	30.6 (29.6–27.8)	29.9 (30.5–28.7)	30.5 (31.6–29.5)	31.5 (32.7–30.2)	31.4 (33.9–30.8)
Ambloplites rupestris (Rockbass)	—[c]	—[c]	23.2 (26.3–21.3)	24.0 (27.2–23.2)	28.4 (28.2–25.1)	28.4 (29.4–26.6)	29.7 (31.0–27.9)	32.2 (32.8–28.9)	30.4 (34.8–29.8)
Catostomidae									
Hypentilium nigricans (Northern hog sucker)	15.3 (12.5–19.8)	20.2 (21.2–15.4)	16.9 (22.8–18.1)	23.0 (24.6–20.6)	27.0 (26.8–23.7)	28.7 (29.2–24.5)	29.4 (32.0–26.1)	28.8 (34.8–27.6)	—[b]
Percidae									
Perca flavescens[a] (Yellow perch)	—[c]	19.2 (19.9–18.5)	20.4 (20.7–19.8)	21.1 (21.8–20.8)	22.4 (28.0–21.6)	—[b]	—[b]	—[b]	—[b]
Salmonidae									
Salmo gairdneri[a] (Rainbow trout)	14.1 (15.7–13.4)	17.1 (17.3–15.7)	18.6 (19.1–17.8)	20.2 (21.1–19.6)	22.2 (23.4–21.2)	—[b]	—[b]	—[b]	—[b]
Salmo trutta[a] (Brown trout)	11.7 (16.2–9.5)	15.5 (17.0–12.4)	17.9 (18.4–14.7)	18.8 (20.8–16.0)	18.5[d] (22.8–16.6)	—[b]	—[b]	—[b]	—[b]
Salvelinus fontinalis[a] (Brook trout)	13.7 (15.0–12.8)	15.2 (16.1–14.5)	17.2 (17.3–16.0)	18.3 (18.8–17.2)	19.0 (20.5–18.2)	—[b]	—[b]	—[b]	—[b]

[a] Fish obtained from state hatcheries.
[b] Acclimation temperature approached or exceeded lethal limits.
[c] No fish available.
[d] Acclimation temperature was 23°C.

formed four different temperature regions (e.g. from $T_1 = 36°C$ and $T_2 = 39°C$ to T_{1-4} = 36, 37, 38, 39°C).

Statistical avoidances were determined only at 3°C increments from a 2-way factorial analysis of variance (Sokal and Rohlf 1969) whereas in the previous study (Cherry et al. 1975), avoidances were obtained at specific 1°C increments. A Duncan's new multiple range test was conducted by utilizing the mean square term from the time spent in the fixed temperature increment with the degrees of freedom from the interaction term, "fish group × temperature interval". A statistical avoidance occurred when significantly ($P = 0.05$) less time was spent at the higher temperature side of the trough relative to that during the control run. A thorough description of the statistical analysis is presented in Stauffer (1975).

7-DAY UPPER LETHAL TEMPERATURE LIMIT

Fish were seined during the highest summer temperature and acclimated upward in the laboratory to their upper lethal temperature limit, the highest temperature at which no mortality occurred during a 7-day period. At maximum summer ambient (27°C) and heated discharge (35°C) temperatures, fish were seined and held in 188-ℓ aquaria (10 fish per aquarium) for 7 days, after which the temperature was increased by 1°C daily to the next acclimation temperature (30°C or 36°C for species captured in ambient or heated areas) and held for 7 days. Control groups of each species were maintained in aquaria at their field temperature. If thermal mortality occurred during a 3°C temperature change, new trials were conducted by raising the temperature 1°C followed by a 7-day holding period.

Results

PREFERRED TEMPERATURES

Temperatures preferred by all species tested in Table 1 increased as water temperatures changed from winter to summer conditions, and except for the telescope shiner (*Notropis telescopus*), yellow perch, and the rainbow, brown, and brook trout, temperature selections exceeded each respective acclimation temperature between 12 to 27°C. Most species selected temperatures lower than their acclimation temperature when tested at 30, 33 or 36°C. Of the 10 species acclimated at 30°C or above, only the fathead minnow (*Pime-*

TABLE 2. Final temperature preferenda of 15 species tested at the Glen Lyn field laboratory calculated by linear and curvilinear regression procedures, with the best statistical fit of the data represented by *(0.05) and **(0.10), "by eye" or connection of data points through a theoretical line with a slope of one, and as reported in the literature.

	Final preferendum (C) obtained at Glen Lyn			
Species	Linear	Curvilinear	"By eye"	Other investigations
Stoneroller	26.2*	26.6	28.8	28.5[a]
Fathead minnow	26.6*	26.2	26.0	—
Bluntnose minnow	28.1*	28.4	29.3	28.4[b]
Rosyface shiner	26.0*	26.1	28.4	27.6[b]
Spotfin shiner	29.4	31.0*	31.9	29.4[b]
Telescpoe shiner	20.0*	21.0	23.6	—
Bluegill	30.9	31.4*	32.1	29.4–31.3,[c] 32.3[d]
Smallmouth bass	30.3	30.3*	31.5	28.0,[d] 31.0[e]
Spotted bass	30.8	31.2*	31.4	24.4[f]
Rockbass	29.0	30.6*	29.8	20.7,[d] 26.8–28.3[c]
Northern hog sucker	26.6	28.6**	29.8	29.2[b]
Yellow perch	21.4*	21.4	22.2	20.2–21.2,[g] 23.3[h]
Rainbow trout	19.2*	19.7	19.8	13.0,[i] 18–19[j]
Brown trout	14.3	17.4*	17.8	12.0,[k] 17.6[d]
Brook trout	15.5*	16.0	16.8	16.0,[l] 19.0[m]

[a] Opuszynski 1971; [b] Cherry et al. 1975; [c] Neill 1971; [d] Ferguson 1958; [e] Barans and Tubb 1973; [f] Dendy 1945; [g] Hile and Juday 1941; [h] McCauley and Read 1973; [i] Garside and Tait 1958; [j] McCauley and Pond 1971; [k] Jammes 1931; [l] Peterson 1973; [m] Creaser 1930.

phales promelas) and bluntnose minnow (*P. notatus*) preferred the highest temperature at the final or highest acclimation level (Table 1). The preferred temperatures for the four species acclimated at 36°C (spotfin shiner, *N. spilopterus*), bluegill, spotted bass (*Micropterus punctulatus*), and rockbass (*Ambloplites rupestris*) were from 0.1 to 2.0°C less than that selected at a lower acclimation temperature (30 or 33°C).

Bluegill and spotted bass consistently preferred the highest temperatures at most levels of acclimation followed by the spotfin shiner and rockbass (Table 1). Of the cyprinids, the telescope shiner followed by the stoneroller (*Campostoma anomalum*) preferred the lowest temperatures. Salmonids generally preferred the lowest temperatures of all species tested with the brown trout consistently selecting the lowest temperatures.

Final temperature preferenda values varied according to the method employed for each species. The highest values for 12 of the 15 species were obtained by connection of the preference data points at the top of the range ("by eye") through the acclimation temperature line with a slope of one (Table 2). Temperature preferenda calculated by curvilinear regression were also higher for 12 of the 15 species when compared to the linear regression procedure (Fig. 1). The fathead minnow was the only species that had the highest final preferendum determined by linear regression, and two species, bluegill and rockbass, had the highest preferendum calculated from the curvilinear regression procedure. From the stepwise regression program, the linear regression model had a significantly (*P* = 0.05) better fit of the preference data for 9 of the 15 species tested, while the curvilinear procedure was the best fit for all but one (rosyface shiner [*N. rubellus*]) of the species acclimated to 33 or 36°C. From either statistical (linear or curvilinear) program used, or from direct data point connection, final preferenda were highest for the bluegill, basses, and spotfin shiner, and least for the trouts.

TEMPERATURES AVOIDED

Upper avoidance temperatures increased with rising acclimation temperatures, but the difference between upper avoidances and the acclimation temperatures was greater at cold acclimation temperatures (12–15°C) than at warmer levels (27–36°C, Table 3). Bluegill, spotted bass, rockbass, and spotfin shiner avoided the highest temperatures at all acclimation levels with the highest avoidance temperature being 37–39°C when acclimated at 36°C. Species that avoided the highest temperatures also had the widest temperature avoidance range, and for most species this range was greater at colder acclimation temperatures (e.g. 18°C difference for spotted bass and

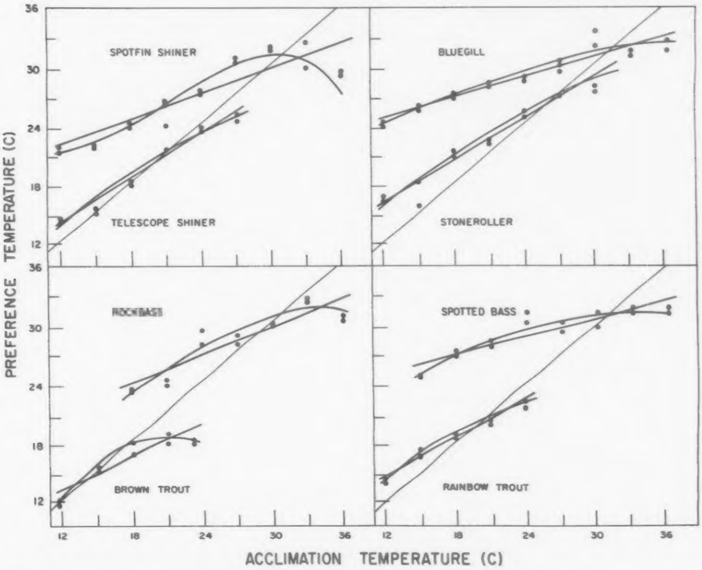

FIG. 1. Final temperature preferenda from linear and curvilinear regressions generated by a stepwise regression procedure.

spotfin shiner acclimated at 18 and 9°C, respectively, and 9–12°C range for both at 36°C). Centrarchids had the widest avoidance ranges, 12–15°C overall, followed by cyprinids with a 9–12°C range (excluding the spotfin shiner), and then by salmonids (9° C).

At the highest acclimation level tested for each species, the upper avoidance temperature either equalled or surpassed the 7-day upper lethal temperature limit by 1–2°C (Table 3). Of the four species tested at 36°C, the statistical upper avoidance occurred at the first temperature increment tested (39°C). The absolute avoidance temperature, which allowed for interaction in four temperature regions (36, 37, 38, 39°C), was 1–2°C less than the statistical avoidance. The four centrarchids tested had the highest 7-day upper lethal temperatures (35–36°C), followed by cyprinids (27°C for the telescope shiner to 36°C for the spotfin shiner, yellow perch (26°C), and salmonids (23–25°C).

Discussion

TEMPERATURE PREFERENCES AND FINAL PREFERENDA

Although increasing acclimation temperatures had a direct effect upon preferred temperatures from winter through spring, preferences declined when acclimation levels above 30°C approached lethal limits (Fig. 1). This was evident for all eurythermal species acclimated at 33 or 36°C but did not occur for fish (except the brown trout) acclimated to 27°C or less (Table 1). At their maximum acclimation temperatures, fish were highly mobile, often required longer orientation periods in the trough, and more variability occurred between replicates than at lower acclimation levels. Other investigators have shown either a leveling off or decline in preferred temperatures as fish approached lethal temperatures (e.g. Pitt et al. 1956 *Cyprinus carpio*; Fry 1947 *Carassius auratus*; Doudoroff 1938 *Girella nigricans*; Brett 1952 *Oncorhynchus nerka*). During falling temperature conditions (Cherry et al. 1975) this trend was not readily apparent, since these higher acclimation temperatures (33, 36°C) were not tested.

The temperature response of the telescope shiner, which was similar to that recorded for trout, was lower than expected as field investigations in the thermally influenced areas indicated higher preferences for the species (Stauffer 1975). This low response may be attributed to the thermal history of the telescope shiner which was seined in a nearby cold, spring-fed stream that received no thermal loading and offered no interaction in a heated plume throughout the year.

Attempts are currently being undertaken to

TABLE 3. Statistical and (in parentheses at the highest acclimation level) absolute upper and lower temperatures avoided, 7-day upper lethal temperature limits, and extreme avoidance temperature ranges of fish, each tested in pairs with four or eight replications, and sampled continuously from the New and East rivers, Virginia, and from state hatcheries at rising acclimation temperatures from 12 to 36°C.

	Acclimation temp (°*C*)									Ranges (degrees) between temp avoided at extreme acclimation temp		7-day Upper lethal temp limit
Species	12	15	18	21	24	27	30	33	36	Lowest	Highest	
C. anomalum[a]	21	24	24	27	30	33	(33)			12	12	31
	9	12	15	18	21	21	21	—[c]	—[c]			
P. promelas[a]	18	24	24	27	30	33	(32)			9	8	—
	9	12	15	15	21	21	24	—[c]	—[c]			
P. notatus	21	24	27	27	27	30	(33)			12	9	32
	9	12	15	18	21	21	24	—[c]	—[c]			
N. rubellus	21	24	21	27	27	33	33	(34)		12	10	33
	9	12	15	15	21	21	21	24	—[c]			
N. spilopterus	27	24	27	27	30	33	36	36	(38)	18	11	36
	9	12	15	18	21	21	24	24	27			
N. telescopus	18	21	24	27	27	(29)				9	11	30
	9	9	15	15	18	18	—[c]	—[c]	—[c]			
L. macrochirus[b]	24	27	30	30	33	36	36	39	(38)	15	11	36
	9	12	15	18	21	21	21	27	27			
M. dolomieui			27	30	33	33	33	(35)		12	8	35
	—[d]	—[d]	15	18	21	24	24	27	—[c]			
M. punctulatus			33	30	33	33	36	39	(38)	18	11	36
	—[d]	—[d]	15	18	21	24	27	27	27			
A. rupestris			27	27	30	33	33	36	(37)	15	10	36
	—[d]	—[d]	12	15	15	18	18	21	27			
H. nigricans[a]			27	30	33	33	33	(34)		15	10	33
	—[d]	—[d]	12	12	15	21	21	24	—[c]			
P. flavescens[b]		21	27	27	(29)					9	11	26
	—[d]	12	15	18	18	—[c]	—[c]	—[c]	—[c]			
S. gairdneri[b]	18	21	21	27	(26)					12	11	25
	6	9	12	12	15	—[c]	—[c]	—[c]	—[c]			
S. trutta[a,b]	15	18	21	24	(25)					9	8	23
	6	9	12	15	17	—[c]	—[c]	—[c]	—[c]			
S. fontinalis[b]	15	18	21	24	(26)					9	8	24
	6	9	15	15	18	—[c]	—[c]	—[c]	—[c]			

[a] Four replicates per acclimation temperature.
[b] Fish obtained from fish hatcheries.
[c] Acclimation temperature approached or exceeded lethal limits.
[d] No fish available.
[e] Acclimation temperature was 23°C.

standardize the method for calculating the final temperature preferendum (C. C. Coutant, Oak Ridge National Laboratory, Tennessee, personal communication). For the same set of preference data, different final preferendum values may be obtained for a species (Table 2). Calculations from the linear regression procedure produced the lowest final preferendum value, although this method generally provided the best fit ($P = 0.05$ level) of the data for species acclimated to 30°C or less. The curvilinear regression model supported the best fit of data for those species where the preferences obtained near or at the highest acclimation temperature were lower than those recorded at a previous acclimation level (Fig. 1). These behavior responses may be attributed to the stressed condition of the fish as acclimation temperatures approached lethal conditions. For preferences that were curvilinear near the top of the range, connection of these points "by eye" may provide the best intersect of the temperature acclimation line with a slope of one. Temperature preferenda determinations are generally highest by this method. Final preferenda from the Glen Lyn species were generally similar to those reported by others except for the high temperature response by rockbass and spotted bass at Glen Lyn. Variations may be attributed to differences in laboratory experimental designs, age of fish, thermal history, and seasonal conditions (Coutant 1975, Meldrim and Gift 1971, Javaid and Anderson 1967, Sullivan and Fisher 1953).

Preferences During Rising and Falling Temperature Conditions

Comparisons of the lineally or curvilineally regressed temperature preferences of each species between falling (Cherry et al. 1975) and rising (this study) temperature conditions showed that only three (bluegill, fathead minnow, and stoneroller) of the nine species studied had significantly ($P = 0.05$) different responses. Differences in temperature preference responses have been reported for fish captured and tested at the field temperature when conditions were declining (summer to winter) from that observed when water temperature was rising from winter to summer (Sullivan and Fisher 1953, Meldrim and Gift 1971). For fish acclimated 18–24 h and then tested, Meldrim and Gift (1971) reported that during rising temperature conditions, temperature preferences were greater than those tested at the same acclimation levels during falling conditions. In Cherry et al. (1975) and the present study, this trend was significant in only one species (fathead minnow), not significantly different in seven others, and in two cases (bluegill and stoneroller), the trend was reversed. Weaknesses exist in the duration of constant holding periods prior to testing. In short holding periods (e.g. 24 h or less), the response of a species may not be indicative of that acclimation temperature. For holding periods of 5–7 days in the present study, the response may be more reflective of the acclimation temperature, but additional variables (artificial food, prolonged stress to laboratory holding conditions) may influence or alter the response.

Avoidances and Lethal Limits

Avoidance temperatures were a function of acclimation temperatures with maximum upper and lower avoidances occurring at the highest and, in some cases, at the final two highest acclimation levels. The difference between the acclimation and upper avoidance temperatures was least at the highest acclimation level, since in most cases the final acclimation temperature was at or near the 7-day upper lethal temperature limit. At final acclimation temperatures (30, 33 or 36°C), the statistical avoidance occurred at the first 3°C increment with the absolute avoidance (first temperature within this increment in which 90% avoidance occurred) being 1–2°C lower (Table 3). Temperature avoidance data are lacking in the literature as seen in the review by Coutant (1975), especially in correlating lethal temperatures with upper avoidances taken at high acclimation levels.

The 7-day upper lethal temperatures reported here for the spotfin shiner, bluegill, spotted bass, and rockbass (36°C), are higher than those reviewed by Brett (1970) and others. In support of these high values and those for the smallmouth bass (35°C), rosyface shiner (33°C), and northern hog sucker (*Hypentilium nigricans*) (33°C), Stauffer (1975) reported that these species were abundant in maximally heated discharges (35°C) of the New River. Since the temperature response of fish is dependent upon its thermal history (Fry 1947), these species may have adapted to the thermal regimes resulting from the operation of the power plant for the past 56 yr. Mosquito fish (*Gambusia affinis*) with a high thermal history at the Savannah River Project (Aiken, South Carolina) have been reported to live at temperatures higher than the critical thermal maxima reported for northern populations (Falke and Smith 1974). Also, in seining fish that inhabit the thermal discharges during maximum ambient summer temperatures in the New River, only the more eurythermal members of each species are sampled and tested which probably tends to inflate the upper lethal temperatures.

Most species tested at their highest acclimation temperature avoided temperatures that just exceeded their 7-day upper lethal temperature limit which was usually equal to the final acclimation temperature (Table 3). Maximum thermal discharges in the New River at Glen Lyn rarely surpassed 35°C, and fish responses at these high temperatures generally were more erratic and variable. Comparison of temperature avoidances to lethal limits at the maximum acclimation level tested was not a reliable indication that fish would avoid sublethal temperatures since fish were probably in a stressed state. Absolute avoidances, which were slightly lower than the statistical avoidance, occurred at temperatures where 50–90% mortality occurred, while temperatures resulting in 100% mortality occurred at the statistical avoidance. The best relationships between upper avoidance, lethal, and 7-day upper lethal temperatures occurred when comparing these values at the acclimation temperature nearest the final temperature preferendum. A fish may interact maximally in its environment when acclimated near or at its final temperature preferendum and also avoid undesirable or lethal temperatures. In most cases, the upper temperature avoided at this acclimation level was less than or equal to the 7-day upper lethal temperature (Tables 1 and 3). As seen in the eurythermal species, final temperature preferenda for all centrarchids and the spotfin shiner were near 30°C and upper avoidances at the 30°C acclimation level did not exceed the 7-day upper lethal temperature. The same tendency was evident for other cyprinids that had final temperature preferenda near 24 or 27°C and for stenothermal species with lower final preferenda. Hence, at this acclimation level fish may have the best ability to actively avoid potentially hazardous lethal temperatures and select a temperature range most conducive to optimal environmental interaction.

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Life Histories of *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson Area, Northwest Territories

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ROSENBERG, D. M., A. P. WIENS, AND O. A. SÆTHER. 1977. Life histories of *Cricotopus (Cricotopus) bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories. J. Fish. Res. Board Can. 34: 247–253.

Cricotopus (*Cricotopus*) *bicinctus* (Meigen) and *C.* (*C.*) *mackenziensis* Oliver emerge from June to September in the Fort Simpson area, Northwest Territories. *Cricotopus bicinctus* begins and ends emerging later than *C. mackenziensis*. *Cricotopus bicinctus* apparently produces a mixture of two and three generations per year, whereas *C. mackenziensis* apparently produces three generations per year. Both species overwinter mainly in the second instar. Emergence data from other studies, when reevaluated, appear to underestimate the number of generations produced by *C. bicinctus*, indicating a similar number of generations as in the Fort Simpson area.

ROSENBERG, D. M., A. P. WIENS, AND O. A. SÆTHER. 1977. Life histories of *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories. J. Fish. Res. Board Can. 34: 247–253.

Cricotopus (*Cricotopus*) *bicinctus* (Meigen) et *Cricotopus* (*Cricotopus*) *mackenziensis* Oliver émergent entre juin et septembre dans la région de Fort Simpson, Territoires du Nord-Ouest. Le début et la fin de l'émergence de *C. bicinctus* ont lieu plus tard que chez *C. mackenziensis*. *C. bicinctus* produit apparemment un mélange de deux et trois générations par année, alors que *C. mackenziensis* produit apparemment trois générations par année. Les deux espèces hivernent en grande partie au stade de second instar. La réévaluation des données sur l'émergence provenant d'autres études indiquent qu'on sous-estimerait le nombre de générations produites par *C. bicinctus* et que le nombre de générations est le même que dans la région de Fort Simpson.

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THE Canada Task Force on Northern Oil Development supported study of the poorly known benthic invertebrate fauna of the Mackenzie Valley, especially in relation to potential environmental impacts caused by development of gas and oil pipelines. Thus, in this paper we describe the life histories of two common species of chironomid before examining these life histories for changes resulting from experimental field exposure to crude oil.

Cricotopus (*Cricotopus*) *bicinctus* (Meigen) was chosen originally because it is common in rivers around Fort Simpson, N.W.T. (Wiens et al. 1975; Rosenberg and Wiens 1976); it was identified as a potentially useful indicator of freshwater contamination by crude oil and petroleum products (Rosenberg and Wiens 1976); and immature forms of *C. bicinctus* are easily identified because they have distinct taxonomic characters (Hirvenoja 1973). Instar analyses have not been done for *C. bicinctus*, despite its Holarctic distribution (Hirvenoja 1973) and its abundance in many locations (Rosenberg and Wiens 1976).

Head capsule measurements of *C. bicinctus* larvae from the Fort Simpson area revealed a bimodality in width-frequency histograms of third and fourth instars which indicated the existence of another species. Subsequent examination of larvae, pupae, and adults confirmed the presence of a new species, *C.* (*C.*) *mackenziensis* Oliver (Oliver 1977).

Materials and Methods

The Fort Simpson area is described in Brunskill et al. (1973). Specimens were collected from the Trail, Martin, Harris, and Jean Marie rivers near the confluence of these rivers with the Mackenzie River. The rivers have similar ranges of physical and chemical water characteristics (Campbell et al. 1975) and are characterized by humic water and riffle-pool sequences. Water depths vary from no flow (Harris River during summers of 1971 and 1972) to >1.5 m (pools in the Martin River). Substrates are generally

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boulders 0.3–1.0 m diam, with silt and finer particles, and fine and coarse sands and gravels. Harris River substrate is mainly friable and fissile chloritic shales with admixtures of silt and finer particles, fine and coarse gravels, and a few boulders <0.5 m diam. Widths of the three larger rivers are 15–20 m during low discharge, whereas the Harris River is usually <10 m. The rivers are covered by ice from October to April. Only the Harris River freezes into the substrate. Ice depths can exceed 2 m (Trail River during winter 1972/1973).

Larvae were collected using artificial substrates (Anderson and Mason 1968) in the Trail and Martin rivers approximately monthly starting in July during the 1972 open-water season and once every 2 mo during ice cover (1972/1973). June, August, and September samples were collected in 1973. Mesh size of nets and sieves used was 200 μm. Sampling details can be found in Brunskill et al. (1973).

Adult insects were collected approximately weekly from all four rivers using Mundie drift-emergence traps (Mundie 1966). Installation was delayed until mid-June because of spring high discharge. Traps were removed when first ice arrived (late September). A rearing program ensured identification of all life stages of *C. bicinctus* and *C. mackenziensis*.

Head capsule widths (as measured halfway between the eyes and posterior median margin) of unmounted third and fourth instar larvae were measured under a binocular microscope at 40× magnification. All larvae in samples with less than 200 specimens were measured. Samples exceeding 200 larvae were quantitatively subsampled to yield at least 200 specimens for measurement. Final data were corrected to yield full sample numbers. Approximately 1500 larvae were measured.

Width-frequency histograms of first and second instar larvae were inadequate to separate the two species and sometimes the two instars as well. Since mean head capsule width of an instar is generally 60% (±10–15%) the mean width of the next higher instar of the same species (Sæther unpublished data), mean head capsule width of third instar *C. mackenziensis*, the smaller of the two species, was used to estimate mean head capsule width of second instars. Specimens at the lower end of the size range would be the smallest second instars found and smaller specimens were assumed to be first instars. This method of instar separation was verified using data given in McCauley (1974) for three other species of *Cricotopus*.

Species of second instar larvae were separated by plotting postmentum length (distance from apex of mentum to posteriomedian margin of head) against ratio of this length to total antennal length. Approximately 200 larvae were slide mounted and measured using a compound microscope at 400× magnification. Because of handling difficulties, numbers of first instar larvae of each species were estimated using proportions obtained for second instars.

Instar data for larvae from the Trail and Martin rivers were combined, where possible, because of the low numbers of individuals of each species. Thus, June and August 1973 data are from the Trail River, July 1972 and February 1973 data are from the Martin River, and August, September, and November 1972 data are combined from both rivers. Insufficient numbers of larvae were collected on September 1973 to be plotted. June 14, 1973 data were plotted as if part of 1972 to facilitate interpretation of life histories of both species. Width-frequency histograms for larvae collected from both rivers at similar times were similar which verified our assumption that life history of a species of chironomid should not vary significantly between nearby, similar rivers. *T*-tests (Snedecor and Cochran 1967) were done on head capsule widths of third and fourth instar larvae of both species in samples collected at similar times to detect significant differences between the two rivers. In all, 10 comparisons were made. Emergence data from all four rivers were also combined because of low numbers of specimens from each river, and a composite graph was prepared using total number of adults emerging over 1 wk, plotted at 3.5 days. Most of the emergence patterns of Chironomidae shown in Ringe (1974) were similar for the same species in two nearby brooks in the Schlitz–Hessen area of Germany so our assumption that species of Chironomidae of neighboring rivers should have similar emergence patterns is probably a safe one.

Results

Cricotopus bicinctus began emerging near the end of June, whereas *C. mackenziensis* began earlier (Fig. 1 and 2). The start of emergence of *C. mackenziensis* could not be determined due to spring high water. Emergence of *C. mackenziensis* ended at the beginning of September, whereas that of *C. bicinctus* lasted until the end of September. Highest water temperatures (15–20°C) occurred in June, July, and August (Fig. 1). Numbers of each sex were approximately equal for both species (49♂♂, 48♀♀ of *C. bicinctus*; 68♂♂, 61♀♀ of *C. mackenziensis*).

Within each instar, *C. bicinctus* larvae were larger than *C. mackenziensis* (Fig. 3).

Only 1 of 10 comparisons of head capsule widths of larvae taken at similar times from each river showed a significant difference in larval sizes (third instar *C. mackenziensis* for August 11 and 15, 1972; $t = 2.921$ for 47 df; $0.005 < P < 0.010$). This significance probably resulted from comparing widely different numbers of specimens having different means but the same variances (Trail River: 11 specimens; mean ± 1 SD = 0.154 ± 0.005 mm; Martin River: 38 specimens; mean ± 1 SD = 0.149 ± 0.005 mm). Similarity of most of the comparisons supported our decision to combine data from the two rivers.

Third and fourth instars of both species dominated in June, July and August (Fig. 1 and 2). First and second instars dominated in September, November, and February. *Cricotopus macken-*

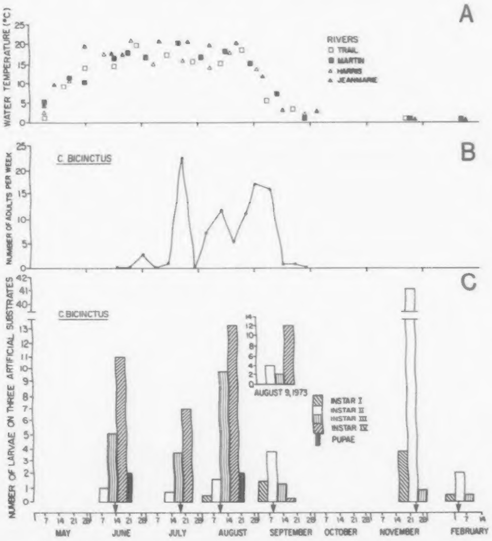

FIG. 1. Water temperatures (degrees Celsius) of the rivers studied (A); numbers of adult *Cricotopus bicinctus* emerging per week (B) and instar distribution and numbers of immature *C. bicinctus* on artificial substrates (C) in Fort Simpson, N.W.T. area rivers. (Dates on horizontal axis are 1972, except June and February which are 1973.)

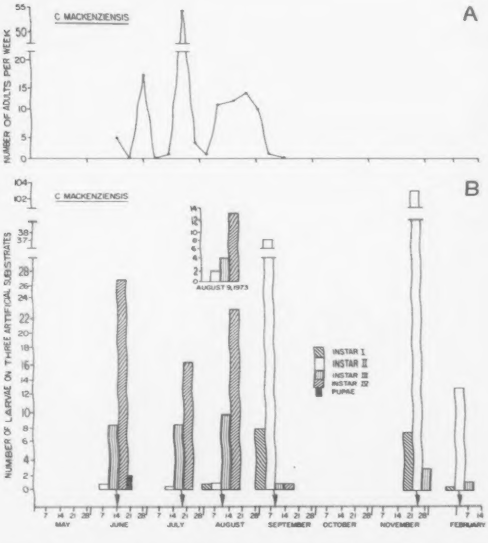

FIG. 2. Numbers of adult *C. mackenziensis* emerging per week (A) and instar distribution and numbers of immature *C. mackenziensis* on artificial substrates (B) in Fort Simpson, N.W.T. area rivers. (Dates on horizontal axis are 1972, except June and February which are 1973.)

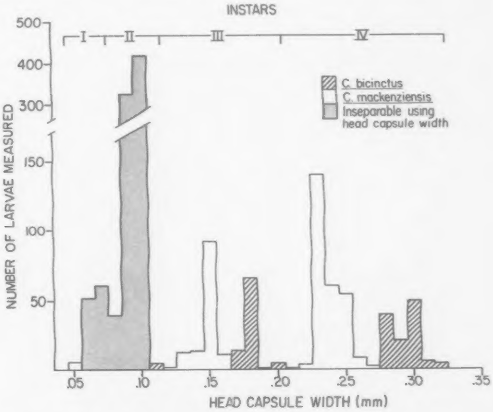

FIG. 3. Distribution of larval head capsule widths of *C. bicinctus* and *C. mackenziensis* from the Fort Simpson area, N.W.T.

ziensis were usually more common than *C. bicinctus*. Both species apparently overwinter mainly as second instars.

Discussion

Figures 1 and 2 indicate that *C. bicinctus* and *C. mackenziensis* have major peaks of emergence in the last week of June, the 3rd wk in July, and 3rd wk in August for *C. mackenziensis*; and in the 3rd wk of July, mid-August, and the beginning of September for *C. bicinctus*. The July peak for both species was mainly due to emergence of 20 *C. bicinctus* and 50 *C. mackenziensis* from the Trail River on July 21, 1972. The longest emergence for *C. bicinctus* recorded in North America was April 23–October 8 from the Thames River, Ontario (Judd 1957). Judd (1960) also recorded *C. bicinctus* emerging as late as mid-October from South Walker Pond, Ont. Fort Simpson populations are, therefore, within the reported emergence period range for *C. bicinctus*. European records also indicate an emergence period from April to October (Hirvenoja 1973). Caution must be used in referring to North American emergence records of *C. bicinctus* (Miller 1941; Judd 1957, 1960, 1962; Buckley and Sublette 1964) because of the possible existence of a *C. bicinctus* species complex in North America (Oliver 1977) and because specimens from these studies were unavailable for confirmation of species determinations (Oliver, Biosystematics Research Institute, Ottawa, personal communication). Also Hirvenoja (1973) reported possible inclusion of *C. intersectus* Staeger in some early European work on *C. bicinctus*. Cumulative percentage emergence data confirm that *C. macken-*

ziensis begins emerging earlier but ends sooner than *C. bicinctus*. Half of *C. bicinctus* emergence occurred by mid-August, whereas half of *C. mackenziensis* emergence was completed almost a month earlier. Emergence of *C. bicinctus* was 98% complete by the first of September, whereas 99% of *C. mackenziensis* emergence occurred prior to August 30.

A number of life histories could possibly explain emergence and larval instar data for *C. bicinctus* and *C. mackenziensis* but only one explains all the available data for each species. Undesirably low numbers of larval instars, pupae, and adults in some samples are a technical difficulty involved in sampling rivers having boulder substrate, extremes in discharge, and severe icing conditions. Higher numbers of specimens would have made interpretation of the life cycles of each species more certain. Unexpectedly, for a subarctic location, more frequent open-water sampling would also have apparently facilitated interpretation.

A 2-yr (or longer) life cycle would require occurrence of third and fourth instars during winter and/or more first and second instars during June, July, and August (Fig. 1 and 2). Also, similarity of August 1972 and 1973 data discounts a 2-yr (or longer) life cycle. Postulation of a 1-yr life cycle with one generation requires explanation for the apparent recruitment of first, second, and third instars in August (Fig. 1 and 2). It would also require an egg aestivation in June and July but not in August or September, an unlikely occurrence since water temperatures were similar from June to August and declined in September (Fig. 1). The first major emergence period of a life cycle with two generations per year would be in the latter part of July because of the reduction in numbers of fourth intsars in July compared with June (Fig. 1 and 2) which fits the emergence graph for *C. bicinctus* (Fig. 1) but not *C. mackenziensis* (Fig. 2). A second emergence period would be in the latter part of August because of the reduction in numbers of late instar larvae and increase in early instars between August and September (Fig. 1 and 2) which fits the emergence graph for *C. mackenziensis* (Fig. 2) but not *C. bicinctus* (Fig. 1). Other emergence peaks for both species would be unexplained. Also, the lack of development of second and third instars from June to July is unexplained (Fig. 1 and 2). Three generations per year requires the assumption that, because of the monthly sampling frequency, we missed collecting high numbers of first and second instars during June, July, and August. Three generations per year would explain the relatively high numbers of third and fourth instars during June, July, and August and the three major emergence peaks apparent for both species (Fig. 1 and 2).

We propose that *C. mackenziensis* has a relatively straightforward life cycle of three generations per year as follows: most of the overwintering larvae have developed by mid-June to fourth instar with low numbers present as seconds and thirds (Fig. 2 and 4). Emergence occurs between mid-June and the end of June although some has occurred previously (Fig. 2). This completes the first generation and eggs laid begin the second generation. By mid-July most of the larvae have developed to fourth instar and proportions of instars resemble those recorded for June (Fig. 2). The second emergence occurs in the latter half of July and eggs laid begin the third generation. Mid-August samples show similar instar composition as the previous 2 mo. Another emergence occurs in the latter half of August to early September. Eggs laid develop to first and second instars by the 1st wk in September. Third and fourth instars in the September sample are larvae from the third generation. November and February samples show that larvae overwinter as second instars. However, some development to third instars is evident (Fig. 2). *Cricotopus* (*Isocladius*) *flavipes* Johannsen also overwinters in second instar (Berg 1950), but *C.* (*Isocladius*) *trifasciatus* (Meigen) overwinters as an egg (Kettisch 1936–1937). Instar data from August 1973 are similar to those of August 1972, indicating that the life cycle of *C. mackenziensis* is synchronized.

For *C. bicinctus* (Fig. 1), we propose that part of the population starts emerging early and completes three generations in 1 yr (solid line, Fig. 5), while the remaining part of the population starts emerging later and completes two generations in the same year (dashed line, Fig. 5). Development of overwintering, early-emerging, fourth instar larvae from June 14[1] (see "IV", far left of Fig. 5) results in the first emergence peak of *C. bicinctus* at the end of June (Fig. 1; solid line, Fig. 5). Brood from this emergence form second and third instars by July 19. Fourth instars on July 19 are late emergers which have developed from second and third instar overwintering larvae present on June 14 (Fig. 1; see "II" and "III", far left of Fig. 5 and dashed line, Fig. 5). First emergence of the late emergers occurs in the 3rd wk in July and, by the August sampling, eggs from this emergence have apparently developed to second and third instars.

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[1] Larval data on June 14 are from 1973. Proportions of instars may differ from a similar date in 1972.

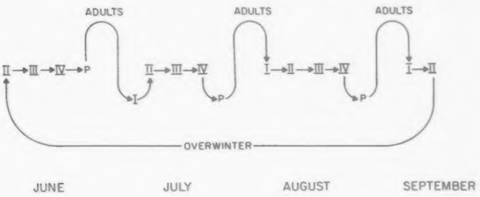

FIG. 4. Proposed schematic life history for *C. mackenziensis.*

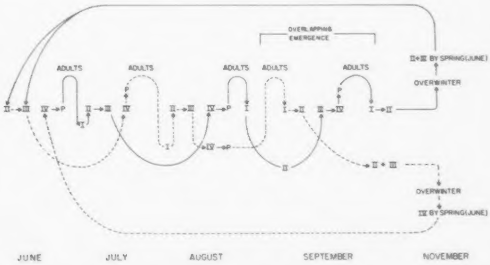

FIG. 5. Proposed schematic life history for *C. bicinctus.* (Solid line = fast emergers; dashed line = slow emergers.)

Meanwhile, brood from the early emergers have reached fourth instar and start emerging in mid-August (Fig. 1; solid line, Fig. 5). By September 7–10 brood from the mid-August emergence has developed to third and fourth instar. The late emergers have reached fourth instar by late August–early September and have emerged, causing part of the final emergence peak (Fig. 1; dashed line, Fig. 5). By November 23 the new brood reaches the second instar plus some thirds which will overwinter, with some development by spring to fourths. The early emergers develop to third and fourth instars by September 7–10 and emerge from early to late September, contributing to the final emergence peak (Fig. 1; solid line Fig. 5). Some of the brood from this emergence develop to second instars which will overwinter, with some development to third instars. The emergence graph and distribution of instars (Fig. 1) indicate that late emergers (two generations in 1972) predominate numerically over the early emergers (three generations in 1972). However, the late emergers will have developed further by spring of 1973 and will be able to complete three generations that year, whereas the early emergers will only complete two, so the numerical dominance will be reversed. Thus, part of the population of *C. bicinctus* has two generations one year, three the next, then two, etc. The remaining part has three generations, then two, then three, etc. Instar proportions should change the following year. Fourth instars should dominate a mid-June 1973 sample which is so (Fig. 1). A mid-July 1973 sample should have more seconds and thirds than fourths and, by August, fourths should dominate with more firsts and seconds than thirds which corresponds with August 9, 1973 (Fig. 1). Two and three generation life cycles likely overlap and, in some years, all *C. bicinctus* may have two or three generations depending on river conditions. Support for this conclusion comes from the overlapping emergence in September and indications that some of the late emergers added a third generation on artificial substrates treated with crude oil (Rosenberg et al. 1977).

A generation time of 4–6 wk for *C. bicinctus* and *C. mackenziensis* is possible because of relatively high water temperatures (15–20°C) and almost total daylight during June, July, and August in the Fort Simpson area. Konstantinov (1958) reported that *Cricotopus algarum* (Kieffer) and *C. sylvestris* (Fabricius) developed from first instars to pupae in about 3 wk at 18°C. Emergence of *C. bicinctus* in more southerly North American latitudes and in Europe lasts from April to October, so, theoretically, four to five generations of 6-wk duration are possible, yet most reports say *C. bicinctus* has either one or two generations (Miller 1941; Thienemann 1954; Darby 1962). *Cricotopus algarum* in Lunzer Untersee, Austria, emerges from May to October and produces three generations per year (Schiemer 1968; based on emergence data and larval instar analysis). In attempting to explain apparent discrepancies in numbers of generations of *C. bicinctus* between Fort Simpson and other locations, we reevaluated other emergence information, only identifying major emergence periods (Table 1). Equating a major emergence period with a generation (we are unable to verify this assumption because other studies have not included larval data), *C. bicinctus* could produce three or more generations in most other locations as well (Table 1). European data show three definite peaks and perhaps up to five. Nearctic data are not as clear. Longer development time for *Chironomus anthracinus* Zetterstedt and *Chironomus plumosus* L. has been reported (Jónasson 1970; Reiss 1968, respectively) from deeper lake waters than shallow, which would explain the difference between shallow and deep water stations in Miller (1941) (Table 1). Also, *C. bicinctus* is usually a shallow-water species, and may be misidentified in Miller (1941) (Oliver personal communication). Emergence of *C. bicinctus* from Medway Creek (Judd 1962) could be interpreted as a single peak but more than one is probably involved because of the long emergence period (early May to early

TABLE 1. Reevaluated emergence data for *Cricotopus bicinctus* from locations other than the Fort Simpson area, N.W.T.

Study	Location	No. of major emergence peaks in year of study
Humphries (1938)	Grosser Plöner See, Ger.	3–5
Meuche (1939)	Grosser Plöner See, Ger.	3–5
Miller (1941)	Costello Lake, Ont.	
	shallow-water stations combined	3
	deep-water station	2
Judd (1957)	Thames River, Ont.	3–5
Judd (1960)	South Walker Pond, Ont.	2–5
Judd (1962)	Medway Creek, Ont.	1–5

September). North American populations of *C. bicinctus* do not appear to have one generation and European populations do not appear to have two generations, as previously claimed. The evidence suggests at least three generations per year in most locations.

An alternating two and three generation per year life cycle for *C. bicinctus* may be normal and widespread or it may be peculiar to the north. If the latter alternative is true, we may be observing a warm-adapted, southern-evolved species that has spread north (Oliver personal communication) and is adjusting a life cycle of 3+ generations per year to fewer in a northern latitude. However, it can produce two generations per year easily. On the other hand, *C. mackenziensis*, which has apparently evolved in and is restricted to northern latitudes (Oliver personal communication), is able to maintain a life cycle with three full generations.

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Responses to Crude Oil Contamination by *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson Area, Northwest Territories

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Cricotopus (*Cricotopus*) *bicinctus* (Meigen) and *C.* (*C.*) *mackenziensis* Oliver, two common species of Chironomidae in the Fort Simpson area, N.W.T., were examined for changes resulting from experimental field exposure to Norman Wells crude oil. Larvae of both species were always present in higher numbers on oiled than unoiled artificial substrates but numbers of *C. bicinctus* increased more quickly than *C. mackenziensis* in response to the oil. *Cricotopus mackenziensis* larvae occurred in higher numbers than *C. bicinctus* larvae on unoiled artificial substrates. *Cricotopus bicinctus* tended to dominate *C. mackenziensis* on oiled artificial substrates. Size of larvae, as measured by head capsule width, was unaffected by increased food supply during periods of maximum algal biomass on oiled artificial substrates. Larvae on oiled artificial substrates apparently produced part of another generation when compared to larvae on unoiled artificial substrates.

ROSENBERG, D. M., A. P. WIENS, AND O. A. SÆTHER. 1977. Responses to crude oil contamination by *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories. J. Fish. Res. Board Can. 34: 254–261.

Nous avons examiné *Cricotopus* (*Cricotopus*) *bicinctus* (Meigen) et *Cricotopus* (*Cricotopus*) *mackenziensis* Oliver, deux espèces communes de Chironomidae de la région de Fort Simpson, T. N.-O., en vue de détecter des changements qui auraient pu se produire à la suite d'une exposition expérimentale sur le terrain à du pétrole brut de Norman Wells. Les larves des deux espèces sont toujours présentes en plus grand nombre dans des substrats artificiels avec pétrole que dans ceux dépourvus de pétrole, mais *C. bicinctus* augmente en nombre plus rapidement que *C. mackenziensis* en réponse au pétrole. Les larves de *C. mackenziensis* se rencontrent en plus grand nombre que celles de *C. bicinctus* dans des substrats artificiels dépourvus de pétrole. *C. bicinctus* tend à dominer *C. mackenziensis* dans les substrats artificiels contenant du pétrole. La taille des larves, mesurée comme largeur de la capsule céphalique, n'est pas affectée par un apport accru de nourriture durant les périodes de biomasse algale maximale sur des substrats artificiels contenant du pétrole. Les larves qui se trouvent sur des substrats artificiels contenant du pétrole produisent apparemment une partie d'une autre génération comparativement aux larves vivant sur des substrats artificiels dépourvus de pétrole.

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THE Canada Task Force on Northern Oil Development supported study of the environmental effects on benthic invertebrates of proposed gas and oil pipelines up the Mackenzie Valley. Previous papers described community and species responses of Chironomidae to crude oil contamination (Rosenberg and Wiens 1976) and life histories of *Cricotopus* (*Cricotopus*) *bicinctus* (Meigen) and *C.* (*C.*) *mackenziensis* Oliver in the Fort Simpson, N.W.T. area (Rosenberg et al. 1977). In this paper, we examine the following aspects of the life histories of larval *C. bicinctus* and *C. mackenziensis* for changes resulting from experimental field exposure to Norman Wells crude oil:

1. Numbers — Higher total numbers of chironomid larvae occurred on oiled artificial substrates than on unoiled (Rosenberg and Wiens 1976). Did *C. bicinctus* and *C. mackenziensis* show the same result? Did oil treatment favor the occurrence of one species over the other?

2. Size — Head capsule size of larval Chironomidae is influenced by temperature and abun-

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TABLE 1. Total numbers of *Cricotopus bicinctus* and *C. mackenziensis* larvae on three oiled and three unoiled artificial substrates in the Trail River, N.W.T.

		C. bicinctus		*C. mackenziensis*	
Date installed	No. days of colonization	oiled	unoiled	oiled	unoiled
July 14, 1972	28	242	18	183	33
	56	2469	12	3127	48
	132	2166	79	4662	647
	335	161	14	365	38
Sept. 8, 1972[a]	279	28	24	77	65
	279	33	13	81	45
July 12, 1973	28	811	18	664	19
	57	47	1	106	2

[a] Two sets of artificial substrates installed.

dance of food (Konstantinov 1958). Oiled artificial substrates supported more algal growth than unoiled (Roeder et al. 1975). Since species of *Cricotopus* are algal grazers (e.g. Thienemann 1954), did more food on the oiled artificial substrates result in larger larvae than on the unoiled substrates?

3. Life cycle — *C. mackenziensis* apparently produces three generations per year, whereas *C. bicinctus* apparently produces a mixture of two and three generations per year (Rosenberg et al. 1977). Did oil treatment alter these life cycles?

Methods

The Trail River study area is described in Rosenberg and Wiens (1976). Larvae were collected using stone-filled basket-type, artificial substrates (Anderson and Mason 1968) which approximated the natural, rocky substrate of the river. Oiled artificial substrates were rolled in a pan of Norman Wells crude, excess oil was allowed to drain, and substrates were installed downstream of untreated controls. Three oiled and three unoiled artificial substrates were removed during each sampling. Installation dates and colonization times of the artificial substrates are shown in Table 1. Further details can be found in Brunskill et al. (1973), Snow and Rosenberg (1975), and Rosenberg and Wiens (1976).

Total numbers of each species and numbers of second, third and fourth instars were counted on oiled and unoiled artificial substrates. Numbers of larvae of each instar were compared as a ratio (*C. bicinctus*:*C. mackenziensis*) to indicate changes in relative occurrence of both species on oiled and unoiled artificial substrates. First instar larvae were omitted because their separation into species was determined by proportions of second instars present (Rosenberg et al. 1977). Samples with >200 larvae were quantitatively subsampled to yield approximately 200 specimens. Subsamples were usually corrected to full sample numbers when numbers of individuals and life cycles were considered.

Mean head capsule width was used to indicate larval size. Measurements of larvae from unoiled artificial substrates in the Trail and Martin rivers were combined (Rosenberg et al. 1977) to provide sufficient numbers for statistical comparison with larvae from oiled artificial substrates. Mean head capsule widths of third and fourth instars of both species were compared using a *t*-test (Snedecor and Cochran 1967) if variances were homogeneous and an adjusted *t*-test (Dixon and Massey 1969) when variances were not homogeneous. The ratio $F = S_1^2/S_2^2$ was used to test homogeneity of variances (Snedecor and Cochran 1967). Twelve comparisons were possible. Overlap of head capsule measurements of larvae of the two species (Rosenberg et al. 1977) prevented a similar analysis of first and second instars.

Information on possible life cycle differences was obtained by comparing frequency histograms of instars on oiled and unoiled artificial substrates for the same dates.

Results

Numbers of larvae of both species were always higher on oiled artificial substrates than unoiled (Table 1). Maximum difference in numbers occurred after 56/57 days of colonization, whereas the minimum difference occurred after 279 days for both species. *C. mackenziensis* occurred in higher numbers than *C. bicinctus* except after 28 days of colonization on oiled artificial substrates. No relationship is apparent between total numbers of either species and amount of oil remaining on rocks of the oiled artificial substrates (Table 1; see also table 1, Rosenberg and Wiens 1976). Instars of *C. mackenziensis* usually occurred in higher numbers than those of *C. bicinctus* on unoiled artificial substrates (Table 2). The difference was greatest in the second instar. Relative occurrence of the two species changed on oiled artificial substrates, *C. bicinctus* instars outnumbering *C. mackenziensis* instars approximately half the time (Table 2). Second instar *C. mackenziensis* continued to outnumber *C. bicinctus*

TABLE 2. Ratios of numbers of *C. bicinctus* to *C. mackenziensis* second, third, and fourth instar larvae on artificial substrates from the Trail and Martin rivers, N.W.T.

Date artificial substrates removed	River	Instar	Numbers of larvae[a]		Approximate ratio
			C. bicinctus	*C. mackenziensis*	*C. bicinctus*:*C. mackenziensis*
			A: Unoiled[b]		
July 19, 1972	Martin	II	—[c]	—[c]	
		III	15	34	1:2
		IV	28	65	1:2
Aug. 11, 1972	Trail	II	—	—	
		III	9	11	1:1
		IV	7	20	1:3
Aug. 17, 1972	Martin	II	—	—	
		III	41	38	1:1
		IV	60	92	1:1.5
Sept. 7, 1972	Trail	II	12	48	1:4
		III	—	—	
		IV	—	—	
Sept. 14, 1972	Martin	II	12	86	1:7
		III	—	—	
		IV	—	—	
Nov. 23, 1972	Trail	II	41	325	1:8
		III	—	—	
		IV	—	—	
June 14, 1973	Trail	II	—	—	
		III	9	26	1:3
		IV	21	80	1:4
			B: Oiled[d]		
Aug. 8, 1972	Trail	II	—[c]	—[c]	
		III	46	25	2:1
		IV	25	23	1:1
Sept. 7, 1972		II	156	368	1:3
		III	53	13	4:1
		IV	69	12	5.5:1
Nov. 23, 1972		II	95	225	1:2
		III	—	—	
		IV	—	—	
June 14, 1973		II	—	—	
		III	5	14	1:3
		IV	50	123	1:2.5
Aug. 9, 1973		II	10	24	1:2.4
		III	49	23	2:1
		IV	23	19	1:1

[a] Not corrected to full sample numbers.
[b] Numbers of specimens on August 9 and September 6, 1973 were too low to use.
[c] Numbers generally $<5\%$ of the total and too low to use.
[d] Numbers of specimens on September 6, 1973 were too low to use.

but the ratios were more equitable than on unoiled artificial substrates.

Mean head capsule widths of third and fourth instar larvae on oiled and unoiled artificial substrates were significantly different in only 4 of the 12 comparisons made (Table 3). Mean head widths of larvae on oiled artificial substrates were larger than those of larvae in unoiled artificial substrates in three of the four significant comparisons.

Proportions of instars usually differed on both types of substrates at similar times (Fig. 1, 2) indicating a difference in larval development time between oiled and unoiled artificial substrates for both species. Comparisons of life cycles of larvae from oiled and unoiled artificial substrates (Fig. 1, 2) suggests that larvae of both species on oiled substrates produced part of another generation (see Discussion).

Discussion

1. NUMBERS

Rosenberg and Wiens (1976) reported that *C. bicinctus* and *C. mackenziensis* (as *C. bicinctus*)

TABLE 3. Comparisons of mean head capsule widths of *C. bicinctus* and *C. mackenziensis* larvae on oiled (O) and unoiled (U) artificial substrates.

				Mean head capsule width (± 1 SD) (*mm*)		Comparisons					
						Instar III			Instar IV		
	Treatment	River(s)	Date of collection	Instar III	Instar IV	*t*-value	DF[a]	*P*	*t*-value	DF[a]	*P*
C. bicinctus	U	Trail & Martin	Aug. 11 & 15, 1972	.176 ± .003(48)[b]	.292 ± .010(67)	0	85	> .900	1.392	34.5	< .200 > .100
	O	Trail	Aug. 11, 1972	.176 ± .004(46)	.296 ± .013(25)						
	U	Trail & Martin	Sept. 7 & 14, 1972	.175 ± .004(11)	—[c]	2.452	30	< .025 > 001[d]	insufficient data for test		
	O	Trail	Sept. 7, 1972	.179 ± .008(53)	.311 ± .022(69)						
	U	Trail	June 14, 1973[e]	.182 ± .009(9)	.294 ± .014(21)	.919	10	> .200	.261	69	> .500
	O	Trail	June 14, 1973[e]	.177 ± .003(3)	.294 ± .015(50)						
	U	Trail	Aug. 9, 1973	.180 (1)	.310 ± .015(5)	insufficient data for test			3.564	26	< .005 > .001[d]
	O	Trail	Aug. 9, 1973	.178 ± .007(49)	.288 ± .012(23)						
C. mackenziensis	U	Trail & Martin	Aug. 11 & 15, 1972	.150 ± .005(49)	.236 ± .009(112)	.942	29.5	> .200	0	24	> .900
	O	Trail	Aug. 11, 1972	.152 ± .010(25)	.236 ± .016(23)						
	U	Trail & Martin	Nov. 23 & 27, 1972	.136 ± .012(12)	—[c]	2.391	20	< .025 > .005[d]	insufficient data for test		
	O	Trail	Nov. 23, 1972	.146 ± .006(10)	.251 (1)						
	U	Trail	June 14, 1973[e]	.148 ± .008(26)	.238 ± .010(80)	1.320	41	> .200	5.570	201	< .005[d]
	O	Trail	June 14, 1973[e]	.151 ± .006(17)	.246 ± .010(123)						
	U	Trail	Aug. 9, 1973	.142 (2)	.241 ± .009(6)	insufficient data for test			0	23	> .900
	O	Trail	Aug. 9, 1973	.147 ± .011(23)	.241 ± .013(19)						

[a] Degrees of freedom ($= n_1 + n_2 - 2$).
[b] Number of larvae measured is in parentheses.
[c] Insufficient data for comparison therefore data from the two rivers not combined.
[d] Significant at the 95% probability level.
[e] Three sets of artificial substrates combined.

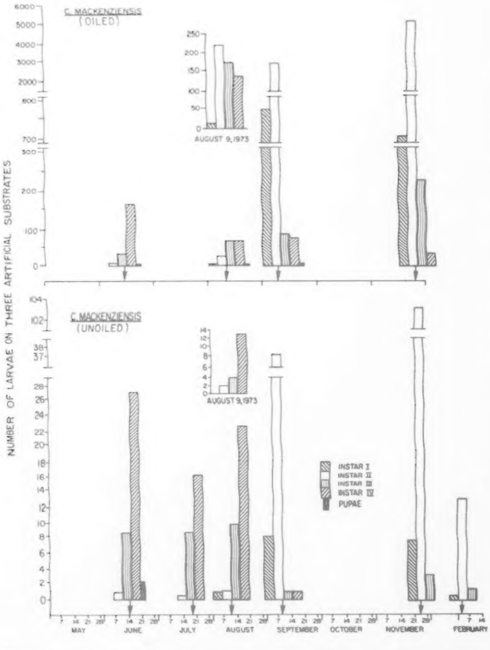

FIG. 1. Instar distribution and numbers of immature *Cricotopus mackenziensis* on oiled artificial substrates (*top*) compared to unoiled artificial substrates (*bottom*). (All dates on horizontal axis are 1972, except June and February which are 1973.)

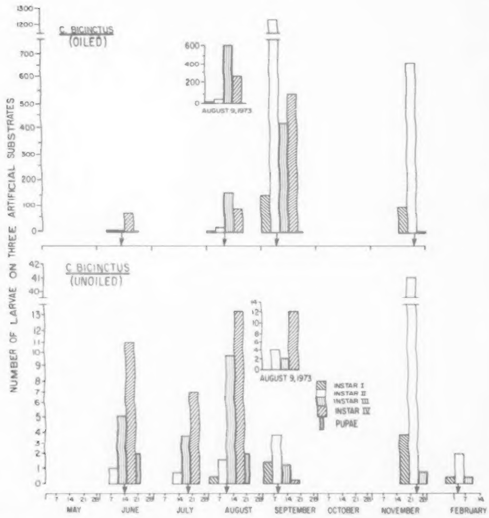

FIG. 2. Instar distribution and numbers of immature *C. bicinctus* on oiled artificial substrates (*top*) compared to unoiled artificial substrates (*bottom*). (All dates on horizontal axis are 1972, except June and February which are 1973.)

showed a strong, positive response to the presence of crude oil and identified *C. bicinctus* as a potential indicator of contamination of fresh waters by crude oil and petroleum products. Our subsequent separation of *C. mackenziensis* from *C. bicinctus* does not affect the conclusions of Rosenberg and Wiens (1976) for *C. bicinctus* itself. Both species were always present in higher numbers on oiled artificial substrates than on unoiled and, in general, *C. mackenziensis* was present in higher numbers than *C. bicinctus* (Table 1). *Cricotopus mackenziensis*, like *C. bicinctus*, would be categorized as showing a Group I, strong positive response to the presence of the crude oil, according to the system used in Rosenberg and Wiens (1976). However, because its zoogeographic distribution and abundance in other freshwater communities are unknown, *C. mackenziensis* would not now be considered useful as an indicator species.

Our observation that *C. mackenziensis* is present in higher numbers than *C. bicinctus* except on oiled artificial substrates after 28 days of colonization (i.e. the first time artificial substrates were removed after being treated) indicates that *C. bicinctus* larvae begin colonizing the oiled artificial substrates before *C. mackenziensis* and are perhaps more tolerant to relatively fresh crude oil. Occurrence of highest numbers of larvae of both species on oiled substrates corresponds, in general, to our field observations of maximum algal growth on the artificial substrates and suggests that these two species of Chironomidae might be responding to the benefits of the presence of the algal community (e.g. amelioration of toxic effects of the crude oil, provision of food and substrate; see Rosenberg and Wiens 1976).

We observed that second to fourth instars of *C. mackenziensis* were numerically dominant over *C. bicinctus* on unoiled artificial substrates but that this dominance changed in favor of *C. bicinctus* on oiled artificial substrates (Table 2). Second instars showed the least change, third instars the most. Thornton and Wilhm (1974) also reported different responses to a toxicant by instars of larval Chironomidae. From existing data we cannot identify the mechanisms underlying this apparent responsiveness of *C. bicinctus* to crude oil. *Cricotopus bicinctus* may be more tolerant of oil residues on the rocks or *C. bicinctus* might be better adapted or more efficient than *C. mackenziensis* at exploiting the increased amount of algae (and bacteria) that develop as a result of oil treatment. Rees (1975), studying substratum preferences of two species of intertidal amphipods (*Gnorimosphaeroma oregonensis oregonensis* [Dana] and *Exosphaeroma amplicauda* [Stimpson]) found that natural substratum preferences (as observed in the field) could be changed with laboratory manipulations of density ratios of the two species. The degree of change depended on the relative numerical strength of the two species. Thus, it is possible that changes in habitat and relative numbers of *C. bicinctus* and *C. mackenziensis* on the oiled artificial substrates confers a competitive advantage on the former in its interaction with the latter.

2. SIZE

Larval head capsule size seemed unaffected during periods of maximum algal biomass on oiled artificial substrates (August and September). Head capsules of larvae from oiled artificial substrates were significantly wider than those of larvae from unoiled substrates on dates other than August and September except for third instar *C. bicinctus* in September 1972 (Table 3). Furthermore, head capsule width of fourth instar *C. bicinctus* from unoiled artificial substrates was significantly wider than from oiled in one August comparison. Zahar (1951) showed that Simuliidae pupae tended to be small during dry summers when food was presumably scarce. Specimens of the early generation of bivoltine species of *Baetis* and Simuliidae are larger than the summer generation (Hynes 1970) and the same observation is true for Chironomidae (e.g. see Rosenberg 1971), perhaps because the early generation has longer to feed. Mean head capsule widths and postmentum (as gular–labial sclerite, in Konstantinov 1958) lengths of third and fourth instar *Chironomus dorsalis* Meigen were larger when the larvae were reared at 10°C than 30°C (Konstantinov 1958). Slower development at the lower temperature may have allowed the larvae to feed longer and grow larger than larvae at the higher temperature. Konstantinov (1958) also demonstrated an increase in postmentum lengths of third and fourth instar *C. dorsalis* with an increase in the amount of food added to the bottom material of the culture. In this study, effects of additional food may not be reflected in increased head size or the effect may not be measurable using our techniques. Measurements related to softer body parts (length, diameter) are probably better indicators of the effects of extra nutrition on chironomid larvae.

3. LIFE CYCLE

The data for *C. mackenziensis* (Fig. 1) can be best explained by postulating faster development of larvae on the oiled artificial substrates. Instar proportions in June 1973 and August 1972 on unoiled artificial substrates indicate that an emergence was about to begin (Rosenberg et al. 1977). Low numbers of larvae on oiled artificial substrates in June 1973 and August 1972, relative to other dates on oiled artificial substrates, indicate that emergence was nearly completed. Reduced numbers of fourth instars compared to thirds on oiled artificial substrates in August 1973 also indicates that emergence was occurring on these substrates prior to that on unoiled controls. Occurrence of fourth instars on oiled artificial substrates in November also indicates faster development than on unoiled artificial substrates of the same date. The data suggest that third instars on oiled artificial substrates in September represent recruitment from what normally would be overwintering seconds. Fourth instars and pupae on oiled artificial substrates in September have reached only third instar on unoiled substrates, and larvae which are present as fourth instars on the unoiled substrates have already emerged from the oiled substrates. By November, the third instars of September on oiled artificial substrates have developed to fourth instars but do not go further because of freezing river conditions. Thus, larvae on oiled artificial substrates almost produce a fourth generation.

The data for *C. bicinctus* (Fig. 2) are also best explained by faster development of larvae on the oiled artificial substrates. However, interpretation is more difficult than for *C. mackenziensis* because of the complex life cycle of *C. bicinctus* (Rosenberg et al. 1977). It appears that all larvae on the oiled artificial substrates produce three generations per year instead of some producing two generations and the rest three. The June data (Fig. 2) indicate development of *C. bicinctus* on oiled artificial substrates is faster than on unoiled because few third instars and many fourths are present. Decreased numbers of fourth instars in August 1972 on oiled artificial substrates compared to unoiled would also indicate faster development on the former, the fourths having emerged. Numbers of fourth instars have also decreased in August 1973 on oiled artificial substrates compared to unoiled controls. Second and third instars on the unoiled artificial substrates have probably developed to thirds and fourths on the oiled artificial substrates. Interpreting faster development for larvae on the September oiled substrates is easier. First and second instars on the unoiled controls, which normally would overwinter as seconds and thirds (= slow emergers; see Rosenberg et al. 1977), have matured to thirds and fourths on the oiled artificial substrates. They emerge and are represented as firsts and seconds on the November oiled artificial substrates. Third and fourth instars which are members of the fast emerging generation (Rosenberg et al. 1977) on unoiled artificial substrates have already emerged from oiled artificial substrates by September and will overwinter as seconds and thirds (November data) instead of firsts and seconds.

The possibility of slower development of larvae of both species on oiled artificial substrates compared to unoiled was discarded because of several differences between postulated occurrences and observed data.

Faster development of both species of *Cricotopus* on the oiled artificial substrates is likely due to the growth of algae on these substrates. Konstantinov (1958) showed that *Chironomus dorsalis* larvae developed faster when more food was available.

Our interpretations depend on the following assumptions:

1. Unoiled artificial substrates represent natural river conditions. Therefore, occurrence of relatively high numbers of larvae on oiled artificial substrates is due to attraction from surrounding natural river substrate or from the drift and is probably caused by an effect of oil treatment, likely algal growth.

2. First or second instar larvae rather than third or fourth instar larvae are attracted to the artificial substrates and remain there to complete their life cycle. First instar Chironomidae are generally planktonic until they find a suitable habitat (Oliver 1971), or until they are ready to molt to second instar at which time they settle (e.g. *C. sylvestris* Fabricus, P. Credland, Bedford College, London, personal communication). Once in a suitable habitat, first or second instar Chironomidae establish a mode of life that usually continues throughout the larval stage (Oliver 1971). Studies on colonization of Plexiglas® artificial substrates by *Cricotopus* spp. larvae showed that initial colonization was mostly by second instars, first instars being mainly planktonic, and that larvae completed their life cycle on the artificial substrates unless severely disturbed (R. Markašová, Charles University, Prague, personal communication). We could not interpret our data by assuming a preferential attraction of later instars by the oiled artificial substrates.

4. CONCLUSIONS

The apparent increase in production of two species of Chironomidae could be considered an aspect of oil treatment beneficial to fish resources. However, *Cricotopus* larvae are small Chironomidae so the overall gain in biomass is probably slight. Furthermore, larvae living within filamentous algae may not be readily available to feeding fish. Finally, the toxic effects of fresh crude oil and the possible development of nuisance algae are not beneficial.

A number of questions arise from this study. Do population structure (i.e. in terms of age and size) and rates of reproduction and death differ in populations of a species exposed to crude oil compared to nonexposed populations? What is the effect of algal growth combined with the presence of crude oil? A life table approach (e.g. Thornton and Wilhm 1975) may be useful in answering these questions. Are rates of feeding, assimilation, respiration, excretion, and other metabolic processes of chironomid larvae significantly affected by exposure to crude oil? How does the presence of algal growth connected with the crude oil affect these processes? Observations we made on slide-mounted gut contents of fourth instar larvae of *C. bicinctus* and *C. mackenziensis* from oiled and unoiled artificial substrates indicated no apparent difference in quantity or quality of algae eaten. However, larvae from oiled artificial substrates usually had oil in their guts and adhering to their parapods and mouth parts. We do not know whether ingestion of the crude oil by *C. bicinctus* and *C. mackenziensis*, most probably an accidental occurrence, is harmful to them metabolically. Thornton and Wilhm (1974, 1975) reported that *Chironomus decorus* Johannsen (as *C. attenuatus* Walker) larvae do not metabolize phenol, a constituent of crude oil. Further research on these questions should be explanatory rather than descriptive in nature.

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Egg and Larval Survival of White Suckers (*Catostomus commersoni*) at Low pH

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TROJNAR, J. R. 1977. Egg and larval survival of white suckers (*Catostomus commersoni*) at low pH. J. Fish. Res. Board Can. 34: 262–266.

White sucker (*Catostomus commersoni*) eggs were incubated in water of high (250 μmhos/cm) and low (20 μmhos/cm) conductivity with nominal pH ranging from 4.2 to 8.0. At pH 4.5 and below in water of low conductivity no eggs survived to hatching. In high conductivity water at pH 4.5 a few prolarvae hatched but were deformed and soon died. At pH 5.0 and above the total hatch was the same at either conductivity level. However, at pH 5.0 there were body deformities and abnormalities in the vascular system and none of the prolarvae survived to the swim-up stage. At both conductivity levels there was a reduction in swim-up at pH 5.3 compared with the control, but none at pH 5.8. Low pH had a delaying influence on the absorption of the yolk sac.

TROJNAR, J. R. 1977. Egg and larval survival of white suckers (*Catostomus commersoni*) at low pH. J. Fish. Res. Board Can. 34: 262–266.

Des œufs de meunier noir (*Catostomus commersoni*) ont été incubés dans de l'eau de haute (250 μmhos/cm) et de basse (20 μmhos/cm) conductivités de pH nominal allant de 4.2 à 8.0. A un pH de 4.5 et moins dans de l'eau de faible conductivité, aucun œuf ne survit jusqu'à l'éclosion. Dans de l'eau de haute conductivité à un pH de 4.5, quelques prolarves éclosent, mais elles sont difformes et meurent peu après. A un pH de 5.0 et plus, l'éclosion totale est la même, quel que soit le niveau de conductivité. Cependant, à un pH de 5.0, on observe des difformités du corps et des anomalies dans l'appareil circulatoire, et aucune des prolarves n'atteint le stade de nage vers le haut. Aux deux niveaux de conductivité, il y a diminution du nombre atteignant le stade de nage vers le haut à un pH de 5.3 comparativement aux témoins, alors qu'il n'y a pas diminution au pH de 5.8. Un bas pH retarde la résorption du sac vitellin.

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DURING the past several years increasing attention has been focused on the high acid levels in precipitation (Likens et al. 1972; Cogbill and Likens 1974). Increased acidification of aquatic ecosystems as a result of atmospheric deposition of strong acids has been identified as the source of declines in fish populations in the LaCloche Mountains of southern Ontario (Beamish and Harvey 1972). Schofield (1976) suggested that increased acidification has also been responsible for the loss of fish populations in the high elevation lakes of the Adirondack Mountains in New York. The affected lakes are typically low in conductivity and alkalinity.

The European Inland Fisheries Advisory Commission (EIFAC 1969) prepared an extensive review of the effects of pH on fish. A large portion of previous experimental research has been conducted at acutely toxic pH levels. These are of some value in comparing tolerance differences between species, but give little insight into the mechanisms involved in the decline and loss of fish populations in natural bodies of water that are gradually becoming more acid. In addition, many of these experiments have been conducted in hard water with no control of CO_2 concentration. The synergistic combination of CO_2 and low pH (Lloyd and Jordan 1964) makes evaluation of the results of these studies impossible on the basis of pH alone. Although the EIFAC report concluded that hardness was of little importance in comparing published data on lethal pH levels, experiments conducted with brook trout (*Salvelinus fontinalis*) (J. R. Trojnar unpublished data) and brown trout fry (*Salmo trutta*) (Leivestad et al. 1976) indicate that it has an ameliorating effect on hydrogen ion toxicity. Consequently, results of experiments conducted in hard water might not be applicable to acid conditions in

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dilute aquatic systems. There is a general lack of experimental information concerning the effects of subacute acid concentrations on egg and fry mortality and reproductive failure in general. Perhaps the most significant finding of the study by Mount (1973) was that reproduction in fathead minnows (*Pimephales promelas*) was affected at much higher pH levels than those which would induce mortality in adult fish.

The white sucker (*Catostomus commersoni*) is widely distributed in the Adirondacks (Greeley and Bishop 1932; Greeley 1934) and field data from the Adirondacks indicate that it is more sensitive to high acidity than the brook trout. Some acidified lakes that formerly contained brook trout and abundant sucker populations of all age-classes now contain only brook trout or have sucker populations dominated by small numbers of large individuals (C. L. Schofield personal communication). Declines in sucker populations could signal the possible demise of the brook trout fishery in problem lakes. Beamish (1972) determined acute toxicity levels of strong acids in hard water for the white sucker; however, the effects of low pH on eggs and larvae have not been evaluated. This study evaluates the effect of low pH during incubation on the hatching success of white sucker eggs and the survival of sucker prolarvae to the swim-up stage.

Materials and methods — Individual incubation units were constructed from plastic (PVC) pipe materials and plexiglass. The units consisted of a trough section of split pipe 25 × 10 × 5 cm glued to a cylindrical section. The latter, 10 cm in diam and 14 cm high, was made of a t-fitting split in half and a section of pipe. The end of the trough and bottom of the cylinder were sealed with plexiglass. Eggs were placed in the trough section of the unit. Water in each individual unit was aerated by air bubbled through tygon tubing. Units were placed in a temperature controlled recirculating water bath and temperature ranged between 17.3 and 19.2°C. Water was acidified with 10 N sulfuric acid to obtain the desired pH levels and was aerated for 24 h to remove excess CO_2. Water was then placed in 76-ℓ plastic containers that delivered water to the incubation units. A section of capillary tubing placed in the tygon tubing going from the plastic containers to each individual incubation unit maintained water flows of approximately 3–4 ml/min.

Hatching success was evaluated at two conductivity levels in combination with pH levels ranging from 4.2 to 8.0. The high conductivity treatment was tap water with a mean conductivity of 253 μmhos/cm (range 231–271). The low conductivity treatment had a mean conductivity of 20.6 μmhos/cm (range 17.2–26.7) and was formed by mixing tap water with demineralized water. A base conductivity of approximately 20 μmhos/cm was chosen because it is representative of what is commonly found in Adirondack waters (C. L. Schofield personal communication). An analysis of the tap water available at the Cornell University Fishery Laboratory where the experiment was conducted was as follows:

Calcium	29.0 mg/ℓ
Magnesium	5.0
Sodium	4.6
Potassium	1.4
Ammonia	.09
Sulphate	26.4
Nitrate	3.16
Chloride	2.4
Bicarbonate	84.9
pH	7.93
Conductivity	231.8 μmhos/cm

Calcium, magnesium, sodium, and potassium were determined by atomic absorption spectroscopy. Ammonia, sulphate, nitrate, and chloride were determined by a Technicon autoanalyzer and bicarbonate by a strong acid grans plot titration. Nominal pH values for both levels of conductivity were 4.2, 4.5, 5.0, 5.4, and 5.8. Controls of nonacidified water were nominally pH 8.0 and 7.0 for high and low conductivity, respectively. The pH of two of three replicates for each treatment was recorded once daily on weekdays. All pH determinations were made with a Fisher Model 420 digital pH/ion meter. Mean pH values and ranges for all nominal treatments are given in Table 1. Conductivity was measured with a Beckman Model RC-19 conductivity bridge and a temperature compensated (25°C) 0.1 K dip cell.

Eggs for the experiments were obtained on June 4, 1975 from the dwarf form of the white sucker (*C.c. utawana* of Greeley and Greene [1931] and Dence [1937]) found in Elk Lake in the northeast Adirondack Mountains of New York (44°02′N, 73°50′W). Elk Lake does not have a low pH problem; values are generally between 6.5 and 7.0. About 25 females and 40 males were involved. These fish were transported to the laboratory where the eggs were fertilized artificially using the dry method (Bagenal and Braum 1971), and then placed in two plastic containers containing nonacidified water of high and low conductivity. The eggs were counted in three replicates of 75 eggs/replicate and placed in individual incubation units containing the acidified water. Eggs in the lower pH were counted first, and the entire process took about 2.5 h from fertilization. Dead eggs, identified by disintegration of the yolk, were enumerated and removed during the incubation period. Based on gross outward appearance, hatched prolarvae were classified as either normal or deformed; Deformed prolarvae ranged from being slightly crescent shaped to corkscrew in shape.

Results — In water of low conductivity there was almost complete mortality at pH 4.16 and 4.48 within 24 h after fertilization, and no eggs survived to hatching (Table 1). At pH 5.02 and above the total hatch was the same ($\alpha = 0.05$, range test). At pH 5.02 there was a reduction in the number of normal prolarvae because of in-

TABLE 1. Mean number of white sucker (*Catostomus commersoni*) eggs successfully hatched after incubation at different pH and conductivity levels and mean number of successful swim-ups. Mean is computed from three replicates of 75 eggs/replicate. Line denotes nonsignificance (∝ = 0.05 range test). Mean deformed not analyzed.

Conductivity (*µmhos*)	During incubation		After hatching		Prolarvae			
	Mean pH	Range	Mean pH	Range	Total	Normal	Deformed	Swim-ups
253	4.20	4.15–4.27	—	—	0	0	0	0
	4.53	4.48–4.61	—	—	11.7	0	11.7	0
	4.97	4.87–5.37	4.95	4.87–5.23	40.7	16.7	24.0	0
	5.41	5.29–5.89	5.32	5.16–5.53	44.7	41.3	3.3	26.7
	5.83	5.67–6.32	5.70	5.54–5.90	46.0	44.7	1.3	36.7
	8.09	8.02–8.15	8.07	7.98–8.20	41.7	41.3	0.3	41.3
20.6	4.16	4.15–4.18	—	—	0	0	0	0
	4.48	4.46–4.55	—	—	0	0	0	0
	5.02	4.91–5.28	4.98	4.89–5.14	33.0	22.3	10.7	0
	5.42	5.33–5.60	5.28	5.16–5.45	38.7	35.0	3.7	18.0
	5.97	5.88–6.15	5.84	5.53–6.19	42.0	40.0	2.0	26.3
	7.12	7.02–7.22	7.10	7.01–7.20	45.3	44.7	0.7	33.7

creased deformities at this pH, and there were no successful swim-ups. There was a significant reduction in swim-ups at pH 5.28 compared with pH 7.10, but no reduction at pH 5.84.

Egg mortality was not as rapid in water of high conductivity at low pH levels. At pH 4.20 there were a few eggs that developed to the eyed stage but none hatched (Table 1). Survival to the eyed stage at pH 4.53 was similar to the higher pH levels; however, few hatched and those that did were severely deformed and died within 24 h. The total hatch was the same at pH 4.97 and above (∝ = 0.05, range test). There was a reduction in normal prolarvae at pH 4.97 and none of the prolarvae survived to swim-up. The number of swim-ups was significantly reduced at pH 5.32 in comparison with pH 8.07, but no reduction occurred at pH 5.70.

A two-way analysis of variance of the number of normal prolarvae with pH and conductivity as the treatments revealed that pH was the only influencing factor (∝ = 0.01). In contrast, a two-way analysis of variance of the effects of conductivity and pH on survival to swim-up revealed a significant (∝ = 0.01) influence of both these variables but there was no significant interaction.

In addition to deformities there were abnormalities in the vascular system at incubation pH 4.98 and 5.02. In some prolarvae there was a reddening of the ventral caudal area. In others the blood appeared to be thickened, and although the heart continued to contract, very little blood was pumped through the system.

Prolarvae in the control groups began to swim-up approximately 5 days after hatching. Lower pH had a delaying influence in the absorption of the yolk sac and prolarvae in these groups generally swam-up several days after the control groups. At posthatching pH 4.95 and 4.98, high and low conductivity, respectively, a few prolarvae made attempts to fill the air bladder but none progressed to the point where they could initiate feeding.

Discussion — Hardness, in this experiment indicated by conductivity differences, had no influence on survival to hatching but did affect survival to swim-up. Leivestad et al. (1976) reported similar findings with brown trout eggs and fry. Packer and Dunson (1970) reported that the active uptake of sodium is inhibited at low pH levels. Long-term exposure of brook trout at pH 4.6 produced reduced blood plasma concentrations of sodium and chloride and this loss of plasma salt is considered to be the primary effect of acid stress (Leivestad et al. 1976). The interrelationships of various salts, such as calcium, with sodium and chloride regulation are not precisely known. The presence of other salts has an ameliorating effect when fry are exposed to low pH so presumably these salts aid in sodium and chloride uptake. The lack of a hardness and pH relationship with eggs has yet to be explained, but may be related to difference in membrane permeability and surface area relationships.

It is likely that recruitment failure can be a major cause in the extinction of fish populations in lakes undergoing acidification. This is suggested by the presence of a few large individuals in some acid Adirondack lakes which formerly had abundant sucker populations of all age-classes and by Mount's (1973) work with fathead minnows in which reproduction was affected at higher pH levels than would cause mortality in adult fish. Two ways in which recruitment failures can occur are through egg and fry mortality and reduced egg deposition.

Acid-induced egg and larval mortality of white suckers in Adirondack waters is a possible source of recruitment failure in lakes subjected to seasonal periods of high acidity, as might be found during the spring snowmelt period. Such decreases in pH have been reported in some Adirondack waters (Schofield 1976). Although one should be hesitant in extrapolating laboratory findings to a field situation, Adirondack lakes that now have remnant populations of white suckers had late June pH values of 4.9–5.2 (project files, unpublished data); these pH levels induced partial or complete egg and larval mortality in the present laboratory experiment. Beamish states the main factor in reproductive failure in the sucker populations he studied was the failure of the female to spawn (Beamish 1974b). Beamish et al. (1975) reported that only 25–35% of the female suckers in George Lake, Ontario, spawned when annual pH levels were between 4.7 and 5.2. This population already experienced a 75% reduction in adult numbers before spawning inhibition occurred. Two years after spawning failure in Lumsden Lake, Ontario, no adult fish were captured (Beamish 1974a, b). These lakes experienced a rapid annual decline in pH within a relatively short period of time which was typical of other LaCloche Mountain lakes (Beamish and Harvey 1972). Rapid annual declines in pH of the magnitude of the Canadian lakes has not been observed in Adirondack lakes (Schofield 1976). No reabsorption of ova has yet been observed in suckers captured in acid waters by Cornell University personnel. Spawning suckers have been reported by local residents in Adirondack lakes that are within the pH range 4.7–5.2 reported by Beamish to induce spawning inhibition. This suggests that Adirondack waters that experience seasonal declines in pH can have recruitment failure through sucker egg and larval mortality. These lakes do not experience the severe annual declines in pH that occur in the LaCloche Mountain lakes and that not only produce spawning inhibition but also adult mortality.

Although suckers will spawn in lakes, a common characteristic is the utilization of tributaries to spawn (Eddy and Underhill 1974). Since streams would tend to recover more rapidly from surface runoff induced acidity (C. L. Schofield personal communication), pH conditions in some streams at the time of spawning could be favorable for a successful hatch. Schofield (1973) presented seasonal pH data for an Adirondack lake and its tributaries which typify this type of spatial variation in pH. In such cases, eggs and fry could have normal survival while in the spawning stream but acid induced mortality could occur upon entering the lake. Although the effect of experiencing a pH change on mortality of swim-up larvae was not intensively investigated in this study, swim-up sucker larvae, which were acclimated for 1 day in water of low conductivity of pH 7.1, exhibited 100% mortality within 3 days when they were transferred to water of low conductivity of pH 4.85.

In long-lived, late-maturing populations, such as suckers, recruitment failure may not become apparent for a number of years. I suggest that if the present rate of acid input into Adirondack lakes does not change appreciably, the extinction of sucker populations may be an insiduous process and by the time there is a noticeable change in the population it may already be close to its demise.

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Rapid Chromosome Preparations from Solid Tissues of Fishes

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KLIGERMAN, A. D., AND S. E. BLOOM. 1977. Rapid chromosome preparations from solid tissues of fishes. J. Fish. Res. Board Can. 34: 266–269.

A technique is described for obtaining well-spread metaphases from solid tissues of fishes without the use of methodologies that rely on tissue grinders, centrifuges, digestive enzymes, or tissue culture. This procedure involves the formation of a cell suspension from acetic alcohol fixed tissues using 50% acetic acid. The suspension is applied to a warm (50°C) slide using a micropipette. Solid tissue preparations may be stained by any of the conventional dyes or treated to reveal Q-bands, C-bands, and nucleolar organizers. Large numbers of slides of fish chromosomes can be made easily and rapidly using this procedure.

KLIGERMAN, A. D., AND S. E. BLOOM. 1977. Rapid chromosome preparations from solid tissues of fishes. J. Fish. Res. Board Can. 34: 266–269.

L'auteur décrit une technique permettant d'obtenir des métaphases bien espacées de tissus solides de poissons sans recourir à des méthodes qui dépendent de hache-tissu, de centrifuges, d'enzymes digestifs ou de culture tissulaire. Le protocole implique la formation d'une suspension de cellules à partir de tissus fixés à l'alcool acétique, utilisant de l'acide acétique à 50%. La suspension est appliquée, à l'aide d'une micropipette, sur une lame chaude (50°C). Les préparations de tissus solides peuvent être colorées avec n'importe quel colorant conventionnel ou traitées de façon à révéler les bandes Q, les bandes C et les organisateurs nucléolaires. Cette méthode permet de préparer facilement et rapidement un grand nombre de lames de chromosomes de poissons.

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FISHES have been the subject of an increasing number of cytogenetic investigations in the areas of systematics, mutagenesis, and aquaculture. However, work has been hampered due to the large number of small chromosomes present in most fish karyotypes and the difficulty in obtaining satisfactory numbers of well spread metaphases from various fish tissues.

Recently, Denton (1973) and Blaxhall (1975) reviewed commonly used methods of preparing slides of fish chromosomes for study. These included sectioning (Nogusa 1960), air drying cells from various fish tissues (Denton and Howell 1969; Howell 1972; Gold 1974), culturing leukocytes (Labat et al. 1967; Heckman and

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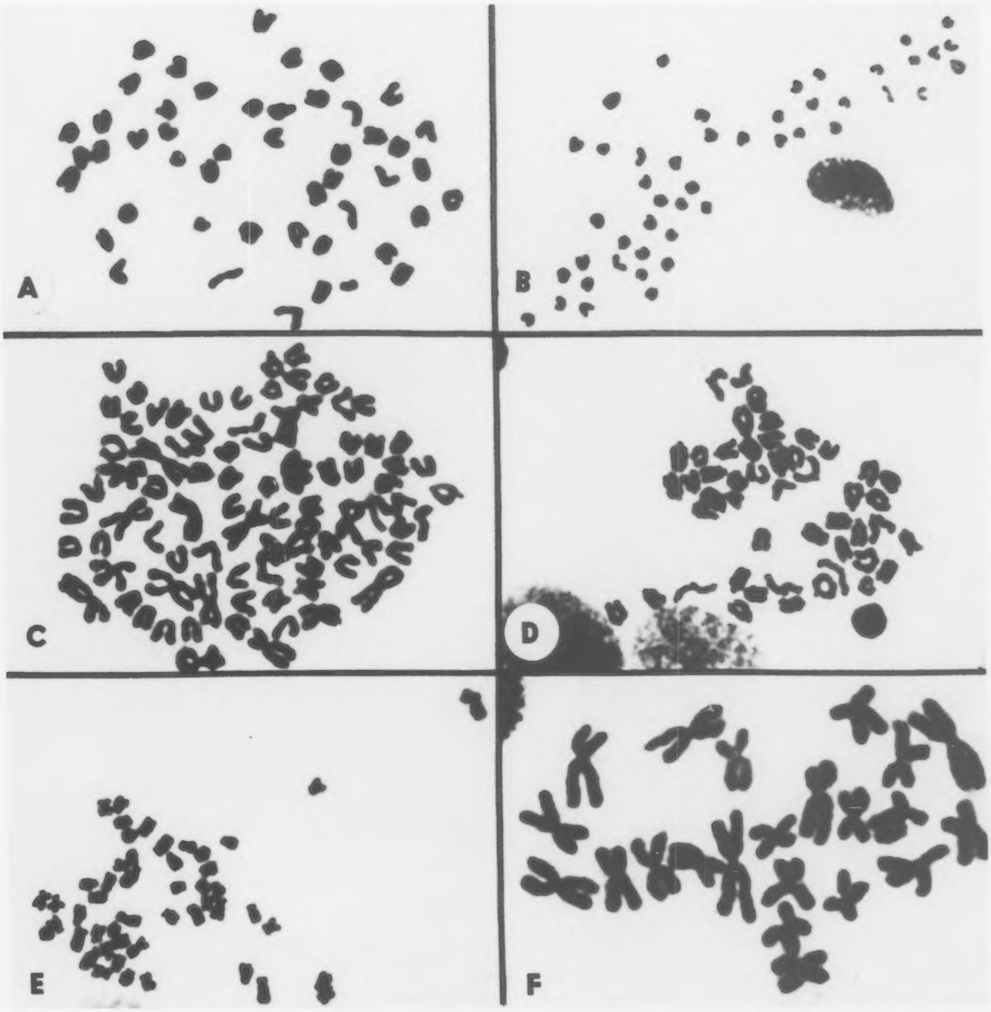

FIG. 1 Metaphases from A, *Xiphophorus helleri*, 2n = 48 (2100×); B, *Poecilia latipinna*, 2n = 46 (1230×); C, *Salvelinus fontinalis*, 2n = 84 (1700×); D, *Lepomis macrochirus*, 2n = 48 (1700×); E, *Rhinichthys atratulus*, 2n = 50 (1275×); F, *Umbra limi*, 2n = 22 (2000×). Metaphases A, B, and C from fry, D from gill epithelium, and E and F from intestinal epithelium.

Brubaker 1970) and fibroblasts (Roberts 1964; Chen 1970, 1975), and squashing testicular tissue, gill epithelium, or blastodiscs (Wright 1955; Roberts 1964; Simon 1964; McPhail and Jones 1966). These methods, although useful in particular instances, all have one or more of the following disadvantages: they are too time consuming, too expensive, or are inappropriate for use in the field.

In this paper we describe an improved method for obtaining good quality metaphases from a variety of fish tissues. Our technique, which is a modification of the solid tissue techniques of Meredith (1969), Evans et al. (1972) and Stock et al. (1972), was originally developed for sister chromatid exchange studies in *Umbra limi* (Kligerman and Bloom 1976). We have now expanded the procedure to obtain preparations from a larger sampling of fish species representing a broad spectrum of chromosome types and numbers.

Materials and methods — Tissue is prepared from fish in the following manner:

1) Allow fishes to swim in a .005–.01% colchicine solution for 6–7 h, or if large enough, inject fish i.p. with 25 μg/g colcemid or colchicine.

2) Sacrifice fish by (a) cutting smaller fish (<1 g wet wt) into two or more pieces, (b) by decapitation; remove gill arches, scales, kidneys, and intestinal tract, and cut them into 0.5-cm pieces where necessary.

3) Place individual tissues into about 10 times their volume of .4% KCl hypotonic solution for 20–30 min.

4) Fix in at least two changes of 3:1 ethanol acetic acid for at least 30 min each. Tissues can then be stored for at least 1 mo at 4°C.

Slides are prepared in the following manner:

1) Remove tissue from fixative and touch to a piece of filter paper to remove excess fixative.

2) Immediately place tissue in well of a depression slide and add 2–3 drops of 50% acetic acid.

3) Mince tissues gently for about 1 min to form a cell suspension. Return unsuspended tissue to fixative.

4) Using a microhematocrit capillary tube (Sherwood BLU-TIP HR1 8889-301506 [75 mm × 1.2 mm] equipped with a rubber bulb, withdraw one capillary tube full of the suspension and expel it onto a clean slide heated to between 40 and 50°C on a slide warmer.

5) Quickly withdraw the suspension back into the capillary tube leaving a ring of cells approximately 1 cm diam on the slide.

6) Repeat steps 4 and 5 producing 2 or 3 rings/slide.

7) Stain slides in 4% Giemsa (or other suitable stain) made up in .01 M phosphate buffer at pH 7.

8) Rinse in distilled water, air dry, and place in xylene for 10 min.

9) Mount in Apochromount, Eukitt, or other suitable mounting media.

The technique may be modified in various ways; all variables can easily be adjusted to meet individual needs.

1) Raising the temperature increases the number of cells near the circumference of the ring and can improve spreading, but high temperatures (>50 C) can interfere with chromosome morphology and cause scattering of the chromosomes.

2) Increased time in acetic acid can improve spreading of chromosomes but can also burst the cells and scatter the chromosomes.

3) Higher concentrations of acetic acid can more rapidly disperse the tissue into a cell suspension but can also lead to poor chromosome morphology.

4) Increasing the time the suspension remains on the hot slide leads to large amounts of poorly spread cells near the circumference, while decreasing the time leads to slides with better spread metaphases but with fewer cells.

5) Increasing the size of the rings usually leads to better spread preparations with some scattering of chromosomes.

Results and discussion — Metaphases from various fish tissues and species, prepared by our modification of the solid tissue technique are shown in Fig. 1. The technique is superior to other fish chromosome methodologies because high quality metaphases can be obtained quickly, and the metaphases can be located easily on the periphery of the rings. In addition, the method is readily adaptable to various banding and staining procedures including Q-bands, C-bands, Ag-bands, and treatment by the FPG technique to reveal sister chromatid exchanges.

Although this method does not offer as many metaphases as leukocyte or fibroblast culture, it is less costly and time consuming. With modifications of the mitotic inhibitor and hypotonic treatment, the solid tissue technique is applicable to amphibians, reptiles, and avian embryos (Bloom and Hsu 1975) as well as fishes and mammals (Meredith 1969; Evans et al. 1972; Stock et al. 1972).

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Nutritional Implications of Surface Feeding by Gizzard Shad (*Dorosoma cepedianum*) on Insect Remains in an Ohio Reservoir

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KING, P. A., T. E. WISSING, E. J. DEVILLEZ, M. J. CHIMNEY, AND W. B. RANDALL JR. 1977. Nutritional implications of surface feeding by gizzard shad (*Dorosoma cepedianum*) on insect remains in an Ohio reservoir. J. Fish. Res. Board Can. 34: 269–273.

On six occasions during July and August 1975, surface feeding by schools of adult gizzard shad (*Dorosoma cepedianum*) on insect remains was observed in Acton Lake, Ohio. Proteinases in the digestive tract presumably hydrolyze the protein in the insect cuticle. The chitin component of the cuticle also has nutritive value, as significant chitinase activity was demonstrated in extracts of gizzard mucosa, hepatopancreas, and intestinal ceca. Values were comparable to activities observed in other fish species. Chitinolytic system activity was not demonstrated.

The mean caloric equivalents of the chironomid and chaoborid exuviae and winged ants in surface debris were 5355, 4363, and 6059 cal/g ash-free dry wt, respectively. Ingestion of insect remains by this forage species could accelerate the return of energy and nutrients in these materials to higher trophic levels.

KING, P. A., T. E. WISSING, E. J. DEVILLEZ, M. J. CHIMNEY, AND W. B. RANDALL JR. 1977. Nutritional implications of surface feeding by gizzard shad (*Dorosoma cepedianum*) on insect remains in an Ohio reservoir. J. Fish. Res. Board Can. 34: 269–273.

A six reprises en juillet et en août 1975, nous avons observé des bancs d'aloses à gésier (*Dorosoma cepedianum*) adultes se nourrissant à la surface de débris d'insectes dans le lac Acton, Ohio. Les protéinases du tractus digestif hydrolisent probablement les protéines de la cuticule des insectes. La chitine présente dans la cuticule a également une valeur nutritive, car nous avons constaté une activité significative de chitinase dans des extraits de muqueuse de gésier, d'hépatopancréas et de cæca intestinaux. Les valeurs sont comparables aux activités observées chez les autres espèces de poissons. Nous n'avons pas démontré d'activité du système chitinolytique.

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Les équivalents caloriques moyens des exuvies de chironomidés et de chaoboridés, et de fourmis ailées dans les débris de surface sont de 5 355, 4 363 et 6 059 cal/g de poids sec sans les cendres, respectivement. L'ingestion de débris d'insectes par cette espèce fourragère serait de nature à accélérer le retour d'énergie et d'éléments nutritifs de ces matériaux vers des niveaux trophiques plus élevés.

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FEW quantitative data are available on the food habits of the gizzard shad (*Dorosoma cepedianum*), a species commonly found in the impoundments and rivers of central and southern USA. The gizzard shad is mainly omnivorous, the diet consisting of phytoplankton, attached algae, microcrustaceans, and organic detritus, which fish obtain by filter feeding, browsing, or both (Ewers and Boesel 1935; Bodola 1956; Kutkuhn 1957; Miller 1960; Price 1963; Dalquest and Peters 1966; Cramer and Marzolf 1970; Jester and Jensen 1972). There is no evidence in the literature to suggest that this species feeds heavily on insect remains at any time during its life cycle.

On six occasions during July 7 to August 20, 1975, we observed large schools of adult gizzard shad feeding on insect debris floating in dense aggregations on the surface of Acton Lake, a 253-ha impoundment in southwestern Ohio. Ingestion of this material raised the question of its potential importance in the nutrition of *D. cepedianum*. In some fish the chitin component of the insect cuticle is apparently digestible, as recent studies have shown significant levels of chitinase activity in 28 of 29 species representing eight orders (Micha et al. 1973) and in four marine teleosts, *Scorpaena porcus*, *Conger conger*, *Boops salpa*, and *Symphodus tinca* (Colin 1972). Bodola (1956) reported, however, that chitinase was lacking in the alimentary tract, hepatopancreas, and gall bladder of the gizzard shad. Protein in the cuticle can presumably be digested by proteinases that are secreted in the digestive tracts of fish (Phillips 1969).

This paper discusses 1) the quantities and caloric equivalents of insect remains in the gizzards of shad taken from schools feeding at the surface, 2) the caloric equivalents of the components of insect debris collected from the surface, and 3) quantitative evidence for chitinase activity in extracts of tissues from the digestive tract.

Materials and methods — During a 20-min period (0820–0840) on July 15, 29 fish were collected at the surface with a DC electroshocker (120 pulses/s) mounted on a pontoon boat. After capture, fish were placed on ice and transported to the laboratory, where total body lengths were measured to the nearest 0.1 cm. Contents of gizzards were removed by incision, examined with a binocular microscope, and, along with the fish remains, oven-dried to constant weight at 105°C.

On July 15, 18, and 23, samples of floating insect remains were taken with a conical plankton net (diam = 30 cm; No. 20 Nitex mesh) towed at the surface. A small amount of material from each sample was preserved in 70% ethanol for identification; the remainder was taken to the laboratory for sorting, drying (105°C for 24 h), and weighing.

The gizzard contents of the shad were combined, and caloric determinations on these and the components of the surface debris were carried out with a Phillipson microbomb calorimeter (Phillipson 1964). The calibration of the instrument is discussed elsewhere (Wissing and Hasler 1968). Prior to combustion, subsamples of the dried materials were enriched with measured quantities of benzoic acid (6.32 cal/mg) and mixed in a dental amalgamator to facilitate pellet preparation. Additional subsamples (5–10 mg) were formed into pellets, placed in tared crucibles, and heated for 3 h in a muffle furnace at 550°C. The ash remaining after ignition was expressed as a percentage of the dry weight of each subsample. The caloric values were expressed as calories per gram of both dry and ash-free weights.

For enzyme studies, adult gizzard shad (ages II–IV; 15–25 cm total length; 30–123 g wet body wt) were collected from June 9 to July 21, 1976. They were immediately placed on ice and subsequently frozen, or extracts were prepared from live specimens within an hour after capture. Tissues examined included the hepatopancreas, gizzard mucosa, and intestinal ceca of frozen and fresh specimens. Each extract was prepared from 30 to 35 fish. Tissue homogenates (except frozen hepatopancreas) were prepared in cold distilled water. The homogenates were maintained at 1°C for 24 h followed by centrifugation at 3000 rpm for 15 min. The supernatant was frozen for 2–3 h prior to assay.

Chitinase assays were performed according to the procedure described by Jeuniaux (1966). Cuttlebone chitin was used as substrate (P-L. Biochemicals Inc. and Calbiochemicals), and the chitobiase solution consisted of 250 mg/ml β-glucosidase (Nutritional Biochemicals, Co.). The acetylglucosamine (AG) released from chitin was measured by the colorimetric method of Reissig et al. (1955). Incubations were at 37°C for up to 180 min with agitation (140 rpm) in a Gyratory Water Bath Shaker Model G76 (New Brunswick Scientific Co. Inc.). Controls consisted of boiled extracts. In assays for the chitinolytic system, the chitobiase solution was replaced with an equivalent volume of distilled water. Activities

TABLE 1. Mean (±SD) chitinase activity in three tissues of fresh and frozen gizzard shad (*Dorosoma cepedianum*). Values are based on 11 assays of five extracts for each of the six preparations.

Tissue	Mean activity (μg AG$\cdot$h$^{-1}\cdot$g^{-1})	
	Fresh	Frozen
Hepatopancreas	185.0(±39.0)	26.2(±36.3)
Gizzard mucosa	202.8(±24.2)	24.2(±33.6)
Intestinal ceca	322.2(±141.0)	100.0(±76.6)

are expressed as micrograms AG liberated per hour per gram of tissue. The minimal chitinolytic activity that can be measured with an accuracy of ±5.0% is approximately 3 μg chitin hydrolyzed$\cdot$h$^{-1}\cdot$ml^{-1} of extract (Jeuniaux 1963). Differences among mean activity values were tested with Duncan's new multiple range test (Steel and Torrie 1960). Significance was declared at the 0.01 probability level.

Results — Of the 29 fish captured on July 15, 21 had substantial quantities of insect debris in their gizzards. The gizzards of the remaining fish were either empty or contained unidentifiable material. The mean (±SE) values of total body length, dry body weight, and dry weight of gizzard contents for the 21 fish containing insect remains were 14.7 (±0.5) cm, 6.50 (±0.34) g, and 0.0111 (±0.0036) g, respectively. A recent age-and-growth study of *D. cepedianum* in Acton Lake indicated that fish in these length and weight ranges are in their fourth growing season (R. J. Pierce, Army Corps of Engineers, N. Atlantic Division, New York, unpublished data).

Examination of the insect remains in surface samples and in fish gizzards revealed large numbers of chironomid and chaoborid exuviae as well as smaller quantities of intact and fragmented winged ants (Hymenoptera: Formicidae). The chaoborid exuviae were derived from *Chaoborus punctipennis*, the only chaoborid species in Acton Lake. No effort was made to identify chironomid exuviae, since a number of species are known to inhabit the lake. Terrestrial winged ants were probably carried out over the lake by wind action and were observed only in the surface samples taken on July 15. The insect debris collected on July 18 and 23 consisted of chaoborid and chironomid exuviae, along with a few ephemeropteran (*Stenonema* sp.?) exuviae.

Analyses of variance revealed that for each tissue tested, the extract from fresh fish demonstrated significantly higher chitinase activity than the corresponding extract from frozen animals (Table 1). For fresh and frozen gizzard shad, extracts of intestinal ceca showed significantly higher activities than extracts of hepatopancreas or gizzard mucosa. There was no significant difference between activities in the gizzard mucosa and the hepatopancreas.

Duplicate assays for the presence of the chitinolytic system were conducted on combined extracts of each of the three tissues from fresh and frozen specimens. None of the six extracts demonstrated significant activity.

The mean calories per gram dry weight values of the chironomid and chaoborid exuviae were very similar (3825 and 3927, respectively, Table 2). The large discrepancy between the calories per gram ash-free dry weight values can be attributed to differences in the mean ash contents of the two kinds of exuviae. Caloric contents of hymenopterans were considerably higher than 5000 cal/g, indicating the presence of appreciable amounts of lipids in the dried tissues of these organisms. The caloric equivalent ($\bar{x} = 3705$ cal/g dry wt) of the combined gizzard contents reflected the relative proportions of exuviae and hymenopterans observed in the gizzards. The ash fraction (32.5% dry wt) in the ingested food probably consists of the inorganic constituents of the insect remains as well as some inorganic material remaining from previous feeding. During times when fish are browsing, the ash component of the gizzard contents often ranges from 80 to 90% of their dry weight (R. J. Pierce unpublished data).

Discussion — Surface feeding by fishes occurs commonly in most freshwater environments, though it is usually confined to living prey items swimming on or just below the surface. The gizzard shad is poorly adapted for this type of feeding behavior, as its mouth is located ventrally in the head region. In contrast, fishes with terminal or dorso-terminal mouths feed very efficiently at the air–water interface. In Acton Lake, surface feeding by *D. cepedianum* is correlated with large emergences of aquatic dipterans and is restricted generally to early morning (0730–0930), when there is little or no wave action.

The ash-free caloric contents of the dipteran

TABLE 2. Caloric values (based on duplicate samples) of the chironomid and chaoborid exuviae, hymenopterans, and the combined gizzard contents (mixed dipteran exuviae and hymenopterans) of 21 gizzard shad taken from Acton Lake, Ohio.

Taxonomic identity	Date(s) collected July 1975	Calories					
		Per g dry wt		Per g ash-free dry wt		Ash (%)	
		Mean	Range	Mean	Range	Mean	Range
Diptera							
Chironomidae (exuviae)	15, 18	3825	3736–3913	5355	5210–5500	28.3	27.9–28.6
Chaoboridae							
Chaoborus punctipennis (exuviae)	23	3927	3899–3956	4363	4332–4395	9.7	9.5–10.0
Hymenoptera							
Formicidae	15	5565	5555–5574	6059	6053–6065	8.5	7.3–9.6
Combined gizzard contents	15	3705	3626–3785	5485	5344–5625	32.5	32.5–32.6

exuviae and hymenopterans reflect the potential energy value of these materials. The insect cuticle is composed chiefly of protein and chitin. In this study, significant levels of chitinase activity were demonstrated in the intestinal ceca, gizzard mucosa, and hepatopancreas, with the intestinal ceca showing the highest activity. The values obtained for *D. cepedianum* are of a magnitude comparable to activities observed in other species of fish where considerable variation has been noted: activities in micrograms AG per hour per gram were 95 in the cecal mucosa and 414 in the intestinal mucosa of the freshwater carnivore, *Perca fluviatilis*; 511 in the intestinal mucosa and 120–450 in the intestinal bulbus mucosa of the stomachless, freshwater omnivore, *Carassius auratus*; and 240 in the cecal mucosa and 120 in the intestinal mucosa of the marine herbivore, *Boops salpa* (Colin 1972; Micha et al. 1973a). Activities of gastric mucosa extracts of fish with stomachs generally display the highest values among tissues tested (14,547 μg AG$\cdot$h$^{-1}\cdot$g^{-1} in *Perca fluviatilis*), and there is less total chitinase activity in extracts from fish where the stomach is reduced, modified, or absent. In general, fish do not show a strong correlation between diet and chitinase synthesis (Jeuniaux 1961a; Jeuniaux 1963; Colin 1972; Micha et al. 1973).

Chitinolytic system activity was not demonstrated in this study. Jeuniaux (1961a, 1961b) has found the activities of chitobiase and the chitinolytic system in vertebrate digestive extracts to be low compared to chitinase activities. Comprehensive comparative studies on the properties of chitobiase are limited, and assays may not have been performed under optimal conditions. We also emphasize that fish obtained for extract preparation had not been feeding on exuviae at time of capture.

The presence of bacteria in gizzard shad extracts cannot be excluded as the source of chitinase activity, and gizzard shad feeding habits do facilitate the intake of large numbers of bacteria. The use of washed gizzard mucosa decreases the possibility of bacterial chitinolytic activity in these extracts. However, an exogenous origin of the enzyme has not been excluded for extracts of the hepatopancreas and, especially, the intestinal ceca.

Commercially treated preparations of chitin were used in this study. Whether shad can hydrolyze insect exuvial chitin was not directly determined, but would presumably include the use of the proteolytic digestive enzyme complement under natural conditions (Phillips 1969).

The browsing and occasional surface feeding exhibited by the gizzard shad in Acton Lake imply considerable flexibility in meeting nutritional requirements. Ingestion of insect remains may also have broader ecological importance. Normally, the exuviae and other insect debris remaining after an emergence period become fragmented and dispersed throughout the water column, with some of the fragments being deposited eventually on the bottom sediments. With time the nutritive value of these particles is reduced through chemical oxidation and the action of various microconsumers. By consuming the insect remains prior to these events, the shad may be able to exploit this food source while it is still concentrated and of high caloric content. Moreover, since *D. cepedianum* is an important forage species in many reservoirs, feeding activity of this sort may provide a pathway for the rapid return of the energy and nutrients in the exuviae to the higher trophic levels of the lake.

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Fecundity of *Balanus balanoides* in Passamaquoddy Bay

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ARNOLD, D. C. 1977. Fecundity of *Balanus balanoides* in Passamaquoddy Bay. J. Fish. Res. Board Can. 34: 273–275.

The fecundity of this common intertidal species has been examined at the point of spawning. The development of reproductive potential by the 2+ year-class, rather than by the 1+ year-class, is confirmed. However, a small reproductive potential was found in the largest members of the 1+ year-class. It is suggested that this capacity could be particularly significant under adverse environmental conditions.

ARNOLD, D. C. 1977. Fecundity of *Balanus balanoides* in Passamaquoddy Bay. J. Fish. Res. Board Can. 34: 273–275.

Nous avons étudié au lieu de ponte la fécondité de cette espèce intertidale commune. Nous confirmons le développement du potentiel reproducteur par la classe d'âge 2+, plutôt que par celle de 1+. Cependant, on trouve un faible potentiel reproducteur chez les plus grands individus

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de la classe d'âge 1+. Cette capacité pourrait avoir une importance particulière dans des conditions adverses du milieu.

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THE nauplii of the common northern barnacle, *Balanus balanoides* (L.), are found in Passamaquoddy Bay most abundantly in April, when they form an important component of the inshore plankton (Legaré and Maclellan, 1960). Studies of adult barnacles from St. Andrews, N.B., have indicated that spawning probably begins in the first week of March and is completed by the first week of May. In the first 12 mo after settlement the adults attain an average basal diameter of 7.5 mm (maximum 9 mm), while in the second growth season an average basal diameter of over 10 mm is reached (Bousfield 1954). Bousfield concluded that the life expectancy is 3–5 yr. Since both adults and larvae feed extensively on the smaller plankters, which also form the food of larvae of commercial species, increased knowledge of this species is of value and assessment of its fecundity at the point of spawning has been considered desirable.

Observations in 1974–76 have shown that at St. Andrews the eggs of *B. balanoides* contain well-developed eyed nauplii at the end of February. Many will hatch when placed in sea water in the laboratory, but only occasional nauplii have been found in inshore plankton samples taken at the same time. In late February 1976, a representative sample of approximately 400 barnacles was collected from a small area at midtide level on the Bar Road, St. Andrews, N.B., and analyzed into millimetre size-classes by measurement of the rostrocarinal diameter, to provide an estimate of the size distribution of the population. The age of barnacles cannot readily be determined directly, but the animals were easily separated into two groups by characteristics of the plates. The majority of smaller specimens, basal diameter 3–9 mm, average 7 mm, had clean white plates with smooth, simple edges and little sign of erosion. The others, including all the largest specimens, basal diameter 4–19 mm, average 9.5 mm, had eroded plates, discolored by algal colonization and pitted by boring organisms, with folded edges. In these the boundaries between plates could not easily be seen. It was concluded that the first group comprised animals completing their 1st yr from settlement, while the others were completing their 2nd or subsequent years. (The few larger animals, 11 mm or more basal diameter, were probably at least 3 yr old.)

About 100 individuals were selected to cover the full range of sizes found in the population. Eggs were removed from the mantle cavity by dissection and dispersed in a known volume of formalinised sea water to prevent hatching. Separation of the eggs is ordinarily difficult, but at the point of spawning it becomes quite easy and use of a special medium to enhance separation (Bagenal, 1966) was not required. Aliquots were removed from the suspension with a Hensen-Stempel pipette and counted in a Bogorov tray. The results showed a variable number of eggs in individuals of a given size-class, but in those judged to be of second or subsequent years the average number increased from 4200 in those containing eggs at 6 mm basal diameter (not all animals at this size contained eggs and there was considerable variation in the number of eggs when present) to 19,000 in one animal of 19 mm basal diameter. The maximum size for this species is stated to be 25 mm basal diameter (Gosner 1971), but such large animals were not found. No significant difference in egg number was noted between the ordinary short body form and some notably taller specimens of comparable basal diameter. The number of eggs found was rather larger than has been reported for members of this species examined in European waters (Barnes and Barnes 1968). However, marine invertebrates often show marked annual fluctuations in their reproductive processes, while racial differences also exist between European and North American populations of this species (Crisp, 1964, 1968).

The percentage abundance of size-classes, average fecundity per size-class, percentage fecundity contributed to the population by each size-class, and the probable relation to year-classes are shown in Fig. 1.

The reproduction of barnacles is well-known to be affected by exposure and tidal level. Although *B. balanoides* has been said to ripen in the 1st yr at low tide levels (Yonge 1949), other studies have indicated that the 1st yr of growth is purely somatic, with spawning occurring first at the end of the 2nd yr (Runnström 1925). This is believed to be true also in Canadian waters (Bousfield 1954). It is of particular interest, therefore, that small numbers of eggs were found in animals which on structural grounds were considered to be members of the first year-class. These were the largest specimens of the white, uneroded forms and contained an average of 200–600 eggs in the size-classes 7–9 mm basal diameter. Eggs were not present in all barnacles

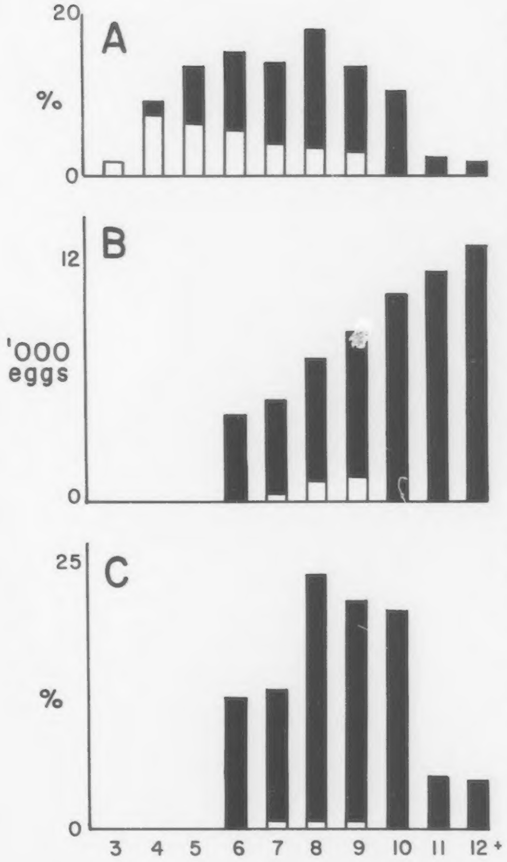

FIG. 1. A, Proportion of millimetre size-classes of *Balanus balanoides*, February–March, 1976; B, average fecundity per mm size class; C, contribution of each size-class to total fecundity of the population. The contribution of the 1+ year-class is shown in white, that of 2+ and later year-classes in black.

of this size and kind, and none at all were found in smaller members of the group. It was also found that the smallest barnacles of the discolored and eroded group, 4–5 mm basal diameter, lacked eggs, though some of this group at 6 mm diameter contained 2000 or more. It thus appears that the development of reproductive capacity in this common Canadian barnacle is not solely dependent upon age, as implied by previous studies, but is instead dependent upon a combination of age and the actual size attained. The present observations show that in Passamaquoddy Bay the critical size for spawning to occur at all is represented by rostrocarinal diameter of 6 mm, but that reproduction at this small size occurs only in the 2nd (or subsequent) yr. However, those animals that attain a basal diameter of 7 mm or more in the 1st yr (the fastest growing members of their year-class) may spawn at the end of that year, though the number of eggs produced per individual is less than 10% of those produced by older individuals of the same size-class. With regard to the distribution of size-classes in the intertidal population, it is obvious that the main reproductive capacity of the Passamaquoddy Bay population depends upon 2nd-yr individuals. It is under adverse conditions, when an unusually high mortality occurs on the shore, that the small reproductive capacity of the larger 1st-yr barnacles, less than 2% of the total fecundity of their respective size-classes in the sample studied, would become important for maintenance of the species in this region.

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Kinetics of Lampricide (TFM, 3-trifluoromethyl-4-nitrophenol) Residues in Model Stream Communities[1,2]

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MAKI, A. W., AND H. E. JOHNSON. 1977. Kinetics of lampricide (TFM, 3-trifluoromethyl-4-nitrophenol) residues in model stream communities. J. Fish. Res. Board Can. 34: 276–281.

The bioconcentration and elimination rates of lamprey larvicide, ^{14}C-TFM (3-trifluoromethyl-4-nitrophenol), were determined for 20 plant and animal species during experimental lamprey control treatments in six indoor model streams. A two-component curve consisting of a rapid initial uptake rate during the first 2 h followed by a reduced linear rate for the remainder of the 24-h exposure best describes the uptake curve for all species examined. The bioconcentration factors (BCF) ranged from 1.1 to 95.5 for animal components and from 0.7 to 12.2 for the plant species. Macroinvertebrate species with soft, relatively permeable integuments accumulate significantly higher residue concentrations (mean BCF = 45.0) than those species with hard chitinized or calcareous exoskeletons (mean BCF = 11.9). Rates of uptake during the linear phase range from 0.36 $\mu g \cdot g^{-1} \cdot h^{-1}$ for the crayfish *Orconectes propinquus* to 17.9 $\mu g \cdot g^{-1} \cdot h^{-1}$ for the caddisfly larvae *Brachycentrus americanus*.

Rates of loss of TFM accumulations correlate with water current and substrate associations. The mean half-life of TFM is 17.8 h for riffle species and 105.9 h for pool-dwelling species. Half-life figures vary from 7.2 h for the crayfish to 5295 h for annelid worms. The longer half-lives observed for several pool species suggest continued accumulation of ^{14}C-labeled residues from the organic matter of the pool bottoms.

MAKI, A. W., AND H. E. JOHNSON. 1977. Kinetics of lampricide (TFM, 3-trifluoromethyl-4-nitrophenol) residues in model stream communities. J. Fish. Res. Board Can. 34: 276–281.

Nous avons déterminé les taux de bioconcentration et d'élimination du larvicide de la lamproie, ^{14}C-TFM (3-trifluorométhyl-4-nitrophénol), chez 20 espèces de plantes et d'animaux au cours d'expériences de contrôle de la lamproie dans six cours d'eau modèles intérieurs. Pour toutes les espèces examinées, une courbe à deux composantes comprenant un rapide taux initial d'assimilation durant les deux premières h, suivi d'un taux linéaire plus lent pour le reste de l'exposition de 24 h est celle qui décrit le mieux le cours de l'assimilation. Les facteurs de bioconcentration (BCF) sont compris entre 1.1 et 95.5 pour les espèces animales et entre 0.7 et 12.2 pour les espèces végétales. Les espèces de macroinvertébrés à téguments mous et relativement perméables accumulent des concentrations de résidus significativement plus élevées (BCF moyen = 45.0) que les espèces à exosquelettes chitinisés ou calcaires durs (BCF moyen = 11.9). Les taux d'assimilation durant la phase linéaire varient entre 0.36 $\mu g \cdot g^{-1} \cdot h^{-1}$ pour l'écrevisse, *Orconectes propinquus*, et 17.9 $\mu g \cdot g^{-1} \cdot h^{-1}$ pour les larves d'éphémère, *Brachycentrus americanus*.

Les taux de perte d'accumulations de TFM sont liés au courant d'eau et aux associations du substrat. La période moyenne de TFM est de 17.8 h pour les espèces vivant dans les radiers et de 105.9 h pour les espèces vivant dans les fosses. La période varie de 7.2 h pour l'écrevisse à 5295 h pour les annélides. Les périodes plus longues observées chez plusieurs espèces vivant dans les fosses donnent à croire qu'il y a accumulation continue de résidus marqués au ^{14}C depuis la matière organique du fond des fosses.

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FROM the early 1960s to the present time over 455,000 kilograms of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) has been applied to Great Lakes tributaries to control larval sea lamprey, *Petromyzon marinus*. The ultimate fate of TFM residues in the aquatic environment is an

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important consideration in evaluating the potential impact of this chemical on nontarget organisms within the treated areas.

A model stream system was used in this study to determine the kinetics of TFM in the water and sediment, and in 17 plant and animal components of the aquatic community. The study was designed to model an actual TFM application for lamprey control where the stream flow is used to dilute and cleanse the exposed area after treatment.

Materials — Six indoor concrete troughs in the state fish hatchery at Paris, Michigan, were modified to maintain model stream communities. Each trough, 8 m long and 0.6 m wide, was equally divided into a 25 cm deep upstream pool and a 8 cm deep downstream riffle. Water was supplied at 100 ℓ/min to each trough from Cheney Creek, a small spring-fed stream adjacent to the hatchery. Natural substrate material and the associated flora and fauna from Cheney Creek were uniformly distributed in each trough. Additional organisms drifting in the entering stream water enhanced the colonization and development of an aquatic community. For a complete description of water quality, stream construction and colonization procedures see Maki and Johnson (1976).

Uniformly ^{14}C-ring labeled TFM (specific activity 3.66 mCi/mM) was used to measure the dynamics of TFM in the model stream communities. Radiochemical purity was ascertained by TLC analysis. An isotope dilution of the ^{14}C-TFM and purified TFM (95% active ingredient, Aldrich Chemical Co.) were prepared in acetone and further diluted in water to provide a concentration of 9.0 mg/ℓ in the stream water. This concentration was chosen, on the basis of acute toxicity data with rainbow trout, *Salmo gairdneri*, and larval silver lamprey, *Ichthyomyzon unicuspis*, as the lampricidal concentration at which Cheney Creek water would be treated under actual field conditions. The final dilution yielded an approximate radioactivity in the stream water of 15.0×10^6 to 20.0×10^6 counts/min per ℓ.

One series of experiments on the accumulation of ^{14}C-TFM was conducted in a series of plexiglass chambers buried in the substrate of each trough prior to colonization. Each chamber consisted of a circular bottom tray (27.9 cm inside diameter × 5 cm deep) buried in and filled with substrate so that the top edge was even with the stream substrate surface. The aquatic community within the tray was enclosed for an experiment by fitting a 10-cm section of plexiglass tubing (30.5 cm inside diameter) over the outside edge of the tray. Thick rubber bands stretched around the bottom tray insured a water tight seal and the chamber top extended above the water surface to prevent exchange with the stream water. Water inside the chamber was circulated by a variable-speed laboratory stirring mechanism.

Methods — The depth of water in a chamber was measured and the exact volume (between 3 and 4.5 ℓ) was calculated before treatment with an isotope dilution of ^{14}C-TFM that provided a concentration of 9.0 mg/ℓ TFM. Samples of the flora and fauna were then removed with forceps at intervals of approximately 1, 3, 5, 8, 14, 20, and 24 h, thoroughly rinsed in water, placed in vials, and immediately placed in a freezer. A 5.0-ml water sample was also removed and frozen at each of the sampling periods.

At the end of a 24-h exposure period the treated water was siphoned from the chamber, the chamber top was removed and the community was exposed to the free flowing stream water. Continued periodic sampling of the exposed community provided data on retention time and elimination of TFM in the various biotic components. This treatment procedure was followed on a biweekly basis from July to January; each run employed a new previously unexposed community. Occasionally species not present in sufficient numbers to permit sampling without depletion were stocked into the chamber just prior to treatment. Usually six to eight species were sampled during a single experiment and three to five replicates of each were tested during the year. During exposure, all organisms and the entire water volume were contained within the chamber by fitting the 10-cm section of plexiglass tubing over the buried chamber bottom. Once the tubing was removed and fresh water allowed to circulate into the chamber for determination of elimination rates, no provisions were made to contain the exposed species. However, the chamber bottom section, protruding up through the substrate, formed an effective cup containing exposed individuals. Drift input of unexposed species to the chamber was also minimized due to the protruding side walls of the buried chamber bottom. In the few isolated instances that this did occur, the lack of detectable residue in the replicate was immediately evident, and the replicate was dropped from statistical analysis.

A second experiment was conducted in which the entire channel of a model stream was treated with ^{14}C-TFM. Water flow into the trough was stopped and the drains were plugged with rubber stoppers. The water in the trough was recirculated by pumping the water from the lower end of the riffle back to the head of the pool section. Air entrained in the pumped water provided reaeration of the stream. Water temperature increased 3°C during the period of recirculation. The stream volume (525 ℓ) was treated with an isotope dilution of 1.0 mCi ^{14}C-TFM and 4.5 g purified TFM dissolved in 10 ml acetone. This dilution provided 8.7 mg/ℓ labeled and unlabeled TFM and an activity of approximately 2.6×10^6 counts/min per ℓ at time 0.

After a 24-h exposure period the treated water was pumped to a large recovery container and the stream water flow was immediately reestablished. Plant, animal, water, and sediment samples were taken during and after exposure in the same manner as in the chamber experiments described above. Therefore, all data represents pooled mean values from the total stream treatment and replicate exposures done throughout July to January in the circular chambers.

Analytical procedure — All plant, animal, and sediment samples were dried at 50°C for 48 h, the weight adjusted to 100–150 mg and combusted to

$^{14}CO_2$ and water in a semiautomated Nuclear Chicago combustion apparatus. Clam and snail whole bodies were dissected free of shells prior to weighing and analysis. The $^{14}CO_2$ was taken up in 10 ml of monoethanolamine–Methyl Cellosolve®, 1:2 (vol/vol). A 2-ml portion was radioassayed with a dual channel Nuclear Chicago Unilux I (Model 6850) liquid scintillation spectrometer in 15 ml of a toluene–Methyl Cellosolve®, 2:1 (vol/vol), fluor mixture. A 2-ml aliquot of each water sample was radioassayed in 15 ml of a toluene–Triton X-100 fluor, 2:1 (vol/vol). Periodic efficiency curves were established for the instrument employing internally quenched standards and all sample counts were converted to disintegrations per minute and microcurie values using the channels ratio and efficiency curve.

Results — Accumulation of TFM residues — The uptake rate of TFM by the total stream community is expressed in Fig. 1 as the reciprocal loss of TFM activity from water during the 24-h exposure periods. Plotted values reflect the mean of nine plexiglass chamber exposures at 9.0 mg/ℓ and the one stream treatment initiated at 8.7 mg/ℓ. The concentration of TFM in water declined rapidly from a mean of 8.9 mg/ℓ to about 8.0 mg/ℓ during the first 2 h after treatment. The slope of the activity curve then declined more slowly reaching 6.9 mg/ℓ after the entire 24-h exposure period.

The graph accurately reflects the reciprocal behavior of the toxicant in the biotic components of the stream. The TFM concentrations in most species demonstrated a rapid accumulation within the first 2 h which corresponds to the rapid loss of activity in the stream water occurring during the same time period. After this initial period, the uptake rates leveled off and uptake increased at a linear rate throughout the duration of the exposure. Since there were generally only two samples taken during the period of rapid uptake, accurate mathematical description of this portion of the curve was not possible.

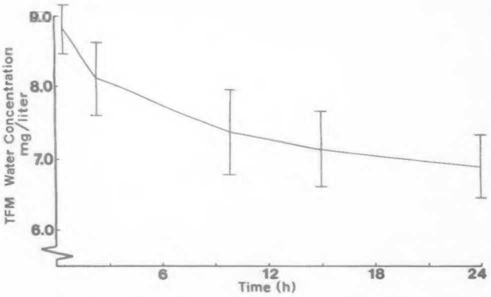

FIG. 1. Uptake of ^{14}C-TFM by organisms expressed as loss from water from initial concentration of 9.0 mg/ℓ. Mean ± 1 SD from 10 replicate treatments.

The uptake rate was expressed as a simple linear regression of micrograms TFM per gram tissue on a dry weight basis against the exposure time in hours. The calculated data yield a y-intercept value which is an estimate of the tissue concentration reached at the end of the initial 2-h exposure period. The linear portion of the uptake curve was described by the regression equation: $y = a + b\ (x)$, where y = concentration of total TFM residue in the organism expressed as micrograms per gram dry weight; a = the y-intercept of regression; b = rate of uptake or slope of the regression; and x = exposure time in hours.

An example of these statistics for the amphipod *Gammarus pseudolimnaeus* is shown in Fig. 2. The data are typical of the uptake dynamics of TFM in each of the biotic components examined. The data taken at 1 and 3 h indicate that the amphipod has a total body residue of 39.5 ± 5.6 and 78.9 ± 21.7 μg/g at these time intervals, respectively. A simple linear regression of all data throughout the 24-h exposure indicates that the y-intercept is 54.6 μg/g with a positive slope of 4.77. The calculation of the y-intercept value thus yields an estimate of the total TFM residue at the end of the initial 2-h period and the equation for the straight line provides prediction and description of the secondary linear phase of uptake. The calculated value of the slope indicates that during this secondary phase, the amphipod accumulates TFM at approximately 4.77 $\mu g \cdot g^{-1}$ (body wt) $\cdot h^{-1}$.

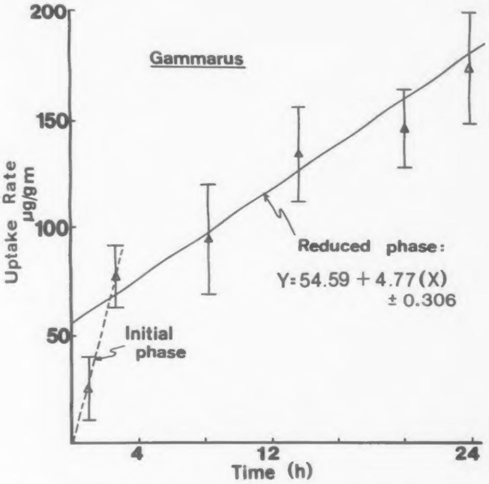

FIG. 2. The rate of uptake of ^{14}C-TFM by the amphipod *Gammarus pseudolimnaeus* during a 24-h exposure to 9.0 mg/ℓ TFM.

TABLE 1. Accumulation, half-life, and rate of loss of total TFM residue in several plant and animal components exposed for 24 h to a mean measured concentration of 8.97 mg/ℓ TFM.

Species	*y*-intercept	Rate of uptake (Slope)	BCF	Initial concn in tissue (μg/g)	Half-life (*h*)	Rate of loss (Slope)
			Pool species			
Annelid worms	222.0	9.7	50.4	426.9	5295.0	−57.3
Isopod						
Asellus militaris	43.2	4.5	16.8	123.0	194.4	−26.9
Amphipod						
Gammarus pseudolimnaeus	54.6	4.8	18.7	176.9	26.2	−62.3
Grayfish						
Orconectes propinquus	1.4	0.4	1.1	11.7	7.2	−6.5
Mayfly						
Hexagenia sp.	31.3	1.9	8.7	86.3	38.6	−27.2
Snail, *Physa* sp.	17.0	2.7	9.2	40.4	23.2	−14.8
Fingernail Clam						
Pisidium sp.	35.0	3.6	13.6	51.1	7.9	−29.9
Green algae, *Cladophora* and *Stigeoclonium*	39.4	0.9	6.8	40.7	65.2	−11.2
Macrophyte						
Ceratophyllum demersum	54.1	2.3	12.2	111.9	437.5	−21.2
Elodea canadensis	21.0	1.2	5.7	35.0	87.4	−9.3
Sediment	13.6	0.9	3.8	27.8	171.7	−6.2
			Riffle species			
Caddisfly,						
Glossosoma sp.	114.6	8.0	34.0	288.2	7.9	−160.4
Limnephilus sp.	95.1	3.3	19.4	106.7	14.0	−46.5
Brachycentrus americanus	129.8	17.9	62.2	550.7	19.5	−213.6
Brachycentrus cases only	189.3	27.9	95.5	464.8	23.8	−168.9
Green algae, *Cladophora* and *Stigeoclonium*	43.9	2.6	11.8	88.7	25.8	−31.4
Moss, *Fontinalis* sp.	27.3	0.5	4.3	20.6	16.3	−8.5
Aufwuchs (5 cm^2) (wet wt)	0.2	0.3	0.7	5.8	24.3	−2.1
Leaf discs (20 mm diam)	43.6	1.8	9.6	44.9	11.0	−21.5

The equation is used to estimate the total residue concentration at any time after the first 2-h exposure by substituting the time of exposure for the value of x in the equation. The bioconcentration factor (BCF) was determined by solving the uptake equation for total body residue after 24-h exposure and dividing this value by the mean initial water concentration of 9.0 mg/ℓ.

Table 1 lists the critical values of linear regressions and bioconcentration factors from pooled data for the various components of the stream examined during the year. Calculated values for the y-intercept which estimate the total body burden at the end of the initial 2-h exposure period range from 1.38 μg/g for young crayfish *Orconectes propinquus* to a high of 221.99 μg/g for annelid worms. Rates of uptake for animal components during the secondary linear phase range from 0.36 $\mu g \cdot g^{-1} \cdot h^{-1}$ for the crayfish to 17.9 $\mu g \cdot g^{-1} \cdot h^{-1}$ for the caddisfly larvae *Brachycentrus americanus*. The general trend evident from these comparisons is that the rate of uptake and therefore the ultimate total body burden of TFM is related to the permeability of the test species' integument. Species with relatively rigid sclerotinized exoskeletons and those with calcareous shells such as the clams and snails accumulated TFM at much slower rates and consequently the total TFM concentration at the end of the 24-h exposure period in these species was significantly lower than in species with relatively soft, membranous integuments.

Brachycentrus americanus and its vegetative cases were analyzed separately to examine variations in total residues. The animals and cases were separated, dried, and combusted as individual samples. The cases and the animals consistently contained high concentrations of TFM. We speculate that the high concentration in the cases may be due to the microflora and microfauna associated with the case. However, 20 mm diam leaf discs placed in the stream several months preceding the exposure did not accumulate high residue levels. Because the leaf discs were relatively well decomposed they may not have supported very dense communities of microorganisms.

Several plants from the model streams were also examined and concentration ranges at the end of the rapid uptake period ranged from 21.0 $\mu g/g$ for *Elodea canadensis* to 54.1 $\mu g/g$ for *Ceratophyllum demersum* in the pool communities. Rates of uptake during the secondary linear phase were generally lower than animal components ranging from 0.5 $\mu g \cdot g^{-1} \cdot h^{-1}$ for the moss *Fontinalis* sp to 2.6 $\mu g \cdot g^{-1} \cdot h^{-1}$ for the filamentous green algae *Cladophora* and *Stigeoclonium* growing together. The bioconcentration factors for stream invertebrates ranged from 1.1 to 95.5 and those for plants ranged from 0.7 to 12.2.

Elimination of TFM residues — The rate of elimination of TFM was calculated as a linear regression of micrograms per gram tissue against the log time in hours. The majority of the total body burden was eliminated rapidly, within the 1st day after exposure, but detectable residues were present in most components for 2–4 wk after exposure. The elimination curve is accurately expressed as a linear equation of the general form: $y = a + (-b)$ ($\log x$); where y = concentration of total TFM residue in the organism expressed as micrograms per gram dry weight; a = the y-intercept of regression or initial concentration in tissue at the initiation of the elimination period; b = rate of loss or slope of regression; and $\log x$ = log of time in hours.

The rate of elimination of ^{14}C-TFM by the amphipod *G. pseudolimnaeus* has been plotted to provide an example of this relationship (Fig. 3). The value for the y-intercept, 176.93 $\mu g/g$, represents the concentration in the tissues of the amphipod immediately following the 24-h exposure period. The rate of loss indicated by the negative slope is 62.35 $\mu g \cdot g^{-1} \cdot (\log\ [h])^{-1} \pm 0.051$. Calculation of the half-life, the time until 50% of the initial TFM residue is eliminated, was done by substituting 50% of the initial body burden as micrograms per gram into the equation for y and solving for x in hours. The half-life for *G. pseudolimnaeus* was found to be 26 h.

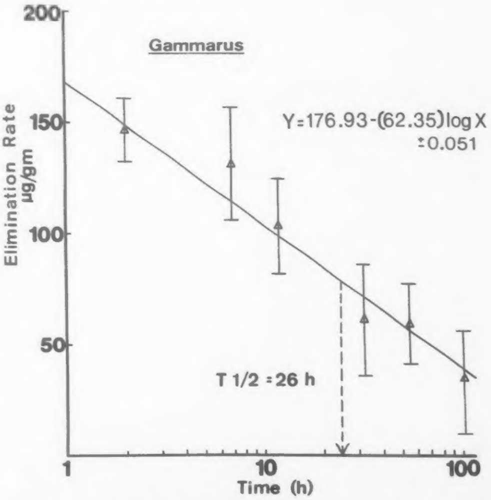

FIG. 3. The rate of elimination of ^{14}C-TFM by the amphipod *G. pseudolimnaeus* following a 24-h exposure to 9.0 mg/ℓ TFM.

The data used to calculate elimination rates and half-life for each of the plant and animal components from pooled data throughout July to January are summarized in Table 1. The half-life calculated for animal species ranged from 7.2 h for the crayfish *Orconectes* to a maximum of 5295 h for annelid worms. The longer half-lives for species inhabiting the pools, e.g. *Asellus militaris* and the annelid worms, probably reflect continued uptake and exchange of TFM or a labeled metabolite from their close association with the organic matter of the pool sediments.

A strong correlation appears to exist between the half-life data and the substrate associations of the test species, i.e. those species closely associated with the organic matter and sediment of the pool communities had significantly longer half-lives than those associated with the gravel and rubble substrate of the riffle communities. The mean half-life calculated for pool species was approximately 105.9 h while the mean for the riffle-dwelling species was only 17.8 h. A comparison of the filamentous green algae *Cladophora* and *Stigeoclonium* from pool and riffle environments demonstrates this difference in elimination rates between the two areas. The half-life calculated from data obtained for algae in the pool area is 65.2 h while the half-life calculated for the same species in the riffle area is only 25.8 h.

The association of the rates of residue elimination with substrate type indicates the accumulation of TFM is largely due to adsorption on the integument of the animal or plant surface. The compound is relatively polar and water soluble and for this reason does not appear to be tightly bound to living tissue in significant concentrations and is rapidly eliminated upon exposure to TFM-free water.

Discussion — The general pattern for accumulation and elimination of TFM residues as determined in our study are compatible with the results of others. Sanders and Walsh (1975) reported an initial rapid uptake and then a slow rate of accumulation was typical for various aquatic insects and crustaceans exposed to sublethal concentrations of TFM. Their data also indicate considerable differences in the rate of uptake and accumulation of TFM by various organisms. Gilderhus et al. (1975) found TFM residues were rapidly eliminated from plants and animals after treatment of the East Au Gres River in Michigan. The more rapid rates of elimination found in their study are probably due to the shorter period of exposure (12 h) and the greater stream flow which would accelerate dilution and dissipation of the residues. In the present study we found a more rapid rate of elimination in the riffle-dwelling species (mean half-life 17.8 h) than in pool-dwelling species (mean half-life 105.9 h).

The rates of uptake and therefore the total body burden of TFM residues after 24 h appeared to be associated with the type of integument of the exposed animals. Those organisms with relatively soft integuments generally accumulated higher residues than those with heavily chitinized exoskeletons or calcareous shells. This observation agrees with toxicity tests (Maki et al. 1975) which indicated generally lower 96 h LC50 values for TFM with soft-bodied invertebrates than those with heavier exoskeletons.

TFM residues do not appear to be a problem in treated watersheds. The relatively rapid elimination of residues indicates little opportunity for transfer between trophic levels. Although the metabolic fate of TFM in aquatic systems is not well known, Kempe (1973) found a progressive decline in concentration and toxicity of TFM in water and lake sediments.

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Occurrence of Piscine Erythrocytic Necrosis (PEN) in the Blood of the Anadromous Alewife, *Alosa pseudoharengus*, from Maine Coastal Streams

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SHERBURNE, S. W. 1977. Occurrence of piscine erythrocytic necrosis (PEN) in the blood of the anadromous alewife, *Alosa pseudoharengus*, from Maine coastal streams. J. Fish. Res. Board Can. 34: 281–286.

Piscine erythrocytic necrosis (PEN) is reported in the blood of anadromous alewives, *Alosa pseudoharengus*, from Maine coastal streams in 1975. This is the first time PEN has been found in any pelagic anadromous fish.

Of 991 alewives collected in 21 samples, 56.1% (371/661) of the prespawning and 10.5% (6/57) of the postspawning alewives had PEN. Within an individual the greatest number of infected red cells was 0.17% (1/600). PEN was not found in 273 juveniles sampled from streams where PEN had been evident in pre- and/or postspawners.

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The difference in infection rates between prespawners, postspawners, and juveniles may be due to either latent or acquired infections. Since alewife PEN resembles that of cod, it may be viral and the possibility exists that other species may be infected.

These results indicate that management practices in which alewives are transferred from one stream to another for the development and maintenance of commercial runs contribute to the spread of PEN.

SHERBURNE, S. W. 1977. Occurrence of piscine erythrocytic necrosis (PEN) in the blood of the anadromous alewife, *Alosa pseudoharengus*, from Maine coastal streams. J. Fish. Res. Board Can. 34: 281–286.

Nous signalons la présence de la nécrose érythrocytique des poissons (NEP) observée en 1975 dans le sang de gaspareaux anadromes, *Alosa pseudoharengus*, de ruisseaux côtiers du Maine. C'est la première fois que la NEP est observée chez des poissons anadromes pélagiques.

Parmi 991 gaspareaux capturés en 21 échantillons, 56.1% (371/661) des gaspareaux qui n'avaient pas encore frayé et 10.5% (6/57) de ceux qui avaient frayé souffraient de la NEP. Chez les jeunes individus, le plus grand nombre de globules rouges infectés était de 0.17% (1/600). Nous n'avons pas observé de NEP chez 273 jeunes d'échantillons provenant de cours d'eau où la NEP avait été observée chez des poissons qui n'avaient pas encore frayé et chez des poissons qui venaient de frayer.

Il est possible que la différence dans les taux d'infection observés chez les poissons avant et après la fraie d'une part et les jeunes d'autre part soit due à des infections latentes ou acquises. Etant donné que la NEP du gaspareau ressemble à celle de la morue, elle peut être causée par un virus, et il se peut que d'autres espèces soient infectées.

Les résultats de cette étude indiquent que les pratiques de gestion en vertu desquelles des gaspareaux sont transférés d'un cours d'eau à l'autre afin de développer et de maintenir des montaisons commerciales contribuent à la propagation de NEP.

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PREVIOUS published findings of piscine erythrocytic necrosis (PEN) have been from benthic nonanadromous species. Laird and Bullock (1969) originally reported PEN in an Atlantic cod, *Gadus morhua*, from Passamaquoddy Bay at St. Andrews, N.B.; in a sea snail, *Liparis atlanticus* (Jordan and Evermann), from Kent Island, N.B.; and from two longhorn sculpins, *Myoxocephalus octodecemspinosus*, from Portsmouth, N.H. Walker (1971) observed characteristic PEN lesions in 2 of 18 Atlantic cod from an aquarium at St. Andrews, N.B., and by electron microscopy confirmed Laird and Bullock's suspicion that PEN was a viral lesion of erythrocytes. Appy et al. (1976) further substantiated the viral characteristics of PEN by comparing light and electron micrographs of infected cells. Appy and Burt (1975) reported PEN in 6.5% (34/523) of Atlantic cod from Passamaquoddy Bay, N.B.

This report documents the findings of PEN in the alewife, *Alosa pseudoharengus*, a pelagic anadromous fish that is under management along the Maine coast. Management practices frequently involve transferring alewives from one stream to another for the development and maintenance of commercial runs. This report indicates that this transfer involves alewives infected with PEN.

Materials and methods — Alewives were dipnetted from 8 streams along the Maine coast between May 14 and November 7, 1975; 991 specimens were collected in 21 samples. Seven of the eight localities (Fig. 1) had established adult alewife runs. Juveniles sampled at Boothbay were progeny of prespawning adults that had been transported to Boothbay from the Damariscotta fishery several months earlier.

Three categories of alewives are considered in this report: 1) prespawning adults ascending fishways or streams to spawn in freshwater; 2) postspawning adults returning to sea; 3) recently hatched juveniles that have spent a month to several months in fresh-

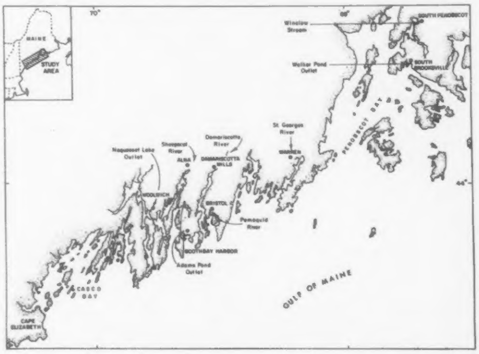


FIG. 1. The central Maine coast indicating localities of the eight streams where blood was collected from alewives, *Alosa pseudoharengus*, to determine the incidence and geographical distribution of PEN.

TABLE 1. The occurrence of PEN in the blood of the anadromous alewife, *Alosa pseudoharengus*, from Maine coastal streams, May 14–November 7, 1975.

Sample source and category	Date	Total no. examined	% PEN incidence	Water temp (°C)[a]	Mean length, SD, and range (*cm*)
Prespawners					
Damariscotta Mills	May 14	73	31.5	11.5	— —
Damariscotta Mills	May 16	148	46.6	14.0	30.0±1.6(26.0–36.5)
Damariscotta Mills	May 20	103	73.8	14.2	29.1±1.3(26.5–32.5)
Damariscotta Mills	May 30	75	48.0	17.0	28.6±1.5(26.5–34.5)
Damariscotta Mills	June 5	25	76.0	17.0	28.5±1.7(25.5–32.5)
Damariscotta Mills	June 12	50	72.0	18.0	28.5±1.5(26.0–32.0)
Damariscotta Mills	June 16	25	68.0	18.0	28.6±1.7(26.0–32.5)
Alna	May 30	60	48.3	17.0	30.7±2.2(25.0–36.0)
Warren	May 30	73	68.5	—	29.3±1.6(26.0–34.0)
S. Penobscot	June 17	22	45.5	17.0	27.0±1.6(24.5–30.0)
Bristol	June 25	7	85.7	24.5	28.4±1.8(27.0–32.0)
Prespawners total		661	56.1		
Postspawners					
Damariscotta Mills	June 24	32	6.3	24.0	28.9±2.0(21.0–32.0)
Woolwich	June 25	25	16.0	24.0	28.9±1.6(26.5–32.0)
Postspawners total		57	10.5		
Juveniles					
Woolwich	Sept. 16	50	00	—	— (6.5–8.5)
Woolwich	Oct. 9	50	00	12.0	8.0±0.2(7.5–9.1)
Damariscotta Mills	Oct. 15	40	00	—	6.0±0.5(5.8–9.0)
Damariscotta Mills	Oct. 16	40	00	—	7.8±0.4(7.2–9.1)
Damariscotta Mills	Oct. 21	25	00	14.5	7.7±0.2(7.1–8.0)
Boothbay	Oct. 22	22	00	13.8	8.4±0.6(7.4–9.4)
S. Brooksville	Oct. 22	21	00	11.7	11.7±0.9(10.3–13.0)
Boothbay	Nov. 7	25	00	10.0	9.0±0.9(8.0–11.2)
Juveniles total		273	00		

[a] Being anadromous, alewives were exposed to other temperatures, perhaps for a longer period of time, than those recorded here.

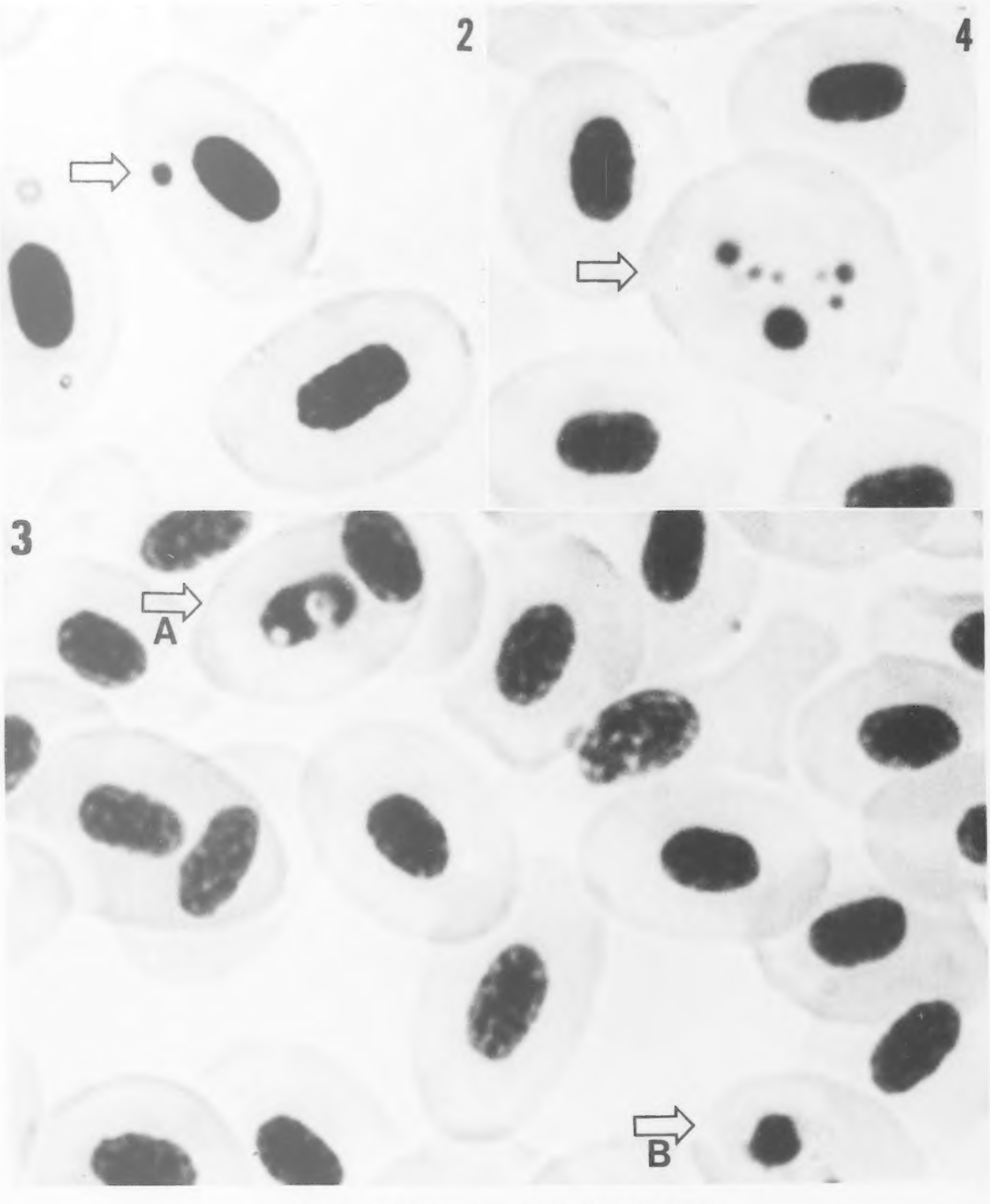
water before venturing to sea. Pre- and postspawning alewives were measured for total length and sexed; juveniles were measured for total length (TL). The 21 samples included 661 prespawning adults (TL 24.5–36.5 cm), 57 post-spawning adults (TL 21.0–32.0 cm), and 273 juveniles (TL 6.5–13.0 cm). Of 588 prespawners examined, 335 (57.0%) were males; of 57 postspawners, 23 (40.4%) were males.

Alewives were not aged in this study, but Bigelow and Schroeder (1953) indicated that alewives are at least 3–4 yr old before returning to freshwater to spawn. Havey (1961) found that 4–6-yr-old alewives dominated spawning runs at Long Pond, Maine. Rounsefell and Stringer (1943) and Walton and Smith (Fisheries Research Station, West Boothbay Harbor, Maine, personal communication) found that alewives in the Damariscotta River are predominantly 4 yr old.

Routine morphological examination of blood obtained from the caudal peduncle of live alewives was made. The caudal peduncle was wiped free of water and mucus with a clean towel, severed, a small drop of blood placed on a clean slide, and a smear made. Air-dried smears were fixed in absolute methanol for 3 min and stained with diluted Giemsa stain for 45 min. Cells were examined under oil immersion at 485 and 970 ×. Each smear was examined for 10 min in the search for characteristic PEN cell lesions.

Results — This is the first time PEN has been found in any pelagic anadromous fish. PEN was initially discovered in alewives on May 14, 1975 in 31.5% (23/73) of the prespawning alewives ascending a fishway at the outlet of Damariscotta Lake at Damariscotta Mills, Maine. No alewives had been examined for evidence of PEN along the Maine coast prior to May, 1975. Further sampling of prespawners at Damariscotta at six intervals from May 16, 1975 to June 16, 1975 (near the end of the spawning run) revealed that 46.6–76.0% of the alewives sampled had characteristic PEN lesions.

Table 1 shows the sampling locations, dates of sampling, and the incidence of PEN at each location. Overall, 56.1% (371/661) of the prespawners from five streams showed evidence of PEN. The disease occurred in postspawning ale-



FIGURES 2–4. Photomicrographs of alewife PEN.

FIG. 2. The cytoplasmic inclusion of the initial stage of infection. The two spots in the cell to the left are artifacts. FIG. 3. A cell in the early stage of infection with seeming vacuolization of the nucleus (A) and a cell in a later stage of infection (B) where the nucleus has apparently fragmented into several particles. FIG. 4. The irregular cell outline, ragged cytoplasm, and the apparent nuclear fragmentation characteristic of some cells in the late stages of infection.

TABLE 2. Comparative cell measurements (50 cells in each case) in microns of infected and uninfected mature erythrocytes from two of the most heavily PEN-affected alewives. Measurements of mature erythrocytes from a PEN-negative alewife are listed for comparison.

Sample source and category	Cytosome Mean length, SD, and range	 Mean width, SD, and range
Uninfected erythrocytes Pemaquid no. 5, 27.5 cm Prespawning female	11.6±0.89 (9.9–13.5)	8.3±0.52 (7.1–9.2)
Infected erythrocytes Pemaquid no. 5	11.4±0.93 (9.2–13.5)	7.8±0.93 (5.0–9.9)
Uninfected erythrocytes Damariscotta no. 39, 28 cm Prespawning male	12.0±0.85 (9.9–13.5)	8.3±0.62 (7.1–9.6)
Infected erythrocytes Damariscotta no. 39	12.1±1.2 (6.4–14.9)	8.0±0.89 (6.4–10.3)
Damariscotta, 28 cm Prespawning female PEN-negative	11.8±0.57 (10.1–13.1)	8.3±0.52 (7.1–9.2)

wives sampled from two streams, but at a reduced level of infection (overall incidence 10.5%). There was no sexual bias towards incidence of infection, nor were any unusual abnormalities or obvious differences in gross parasitism observed between infected and uninfected alewives. PEN was not found in 273 juveniles sampled from four streams, including those from Damariscotta and Woolwich, where PEN had been found in pre- and/or postspawners.

Although prespawners showed a relatively high incidence of PEN as a group, individuals were only lightly infected. The typical individual red cell infection was 0.02% whereas the heaviest was 0.17%. Similar low rates of infection were found in individual pre- and postspawners.

The infected erythrocytes in alewives resembled the characteristic patterns of PEN illustrated by Laird and Bullock (1969) for cod. Immature erythrocytes were rarely infected. As a rule, characteristic nuclear distortion was present without a visible cytoplasmic inclusion. When inclusions were present, they were more often found in cells apparently in the initial stages of infection and in the most heavily infected alewives (Fig. 2). Apparent vacuolization of the nucleus was evident in the early stages of infection (Fig. 3). Cell membranes of infected cells were usually intact though irregular cell outlines and ragged cytoplasm were observed in some instances (Fig. 4).

Within two of the most heavily diseased alewives, the infected erythrocytes had a greater overall size range but somewhat smaller average individual dimensions than uninfected erythrocytes (Table 2).

Discussion — The difference in infection rates between prespawners, postspawners, and juveniles may be due to either latent or acquired infections. The higher incidence of PEN in prespawners may be associated with the activation of a latent infection due to stresses incurred on the spawning run. Postspawners, generally believed to be in worse physiological condition than prespawners, could be expected to have heavier individual infections. However, the most heavily infected alewives may not have been sampled in this study if those individuals did not successfully compete for space in the fishways or were unable to return to the fishways after spawning.

Since alewife PEN resembles that of cod, it may be viral. If individuals are found with 5–10% of their blood cells infected then examination by electron microscopy will be feasible. Search for these individuals in natural and captive populations is underway.

If this infection is viral, the possibility exists that the virus could be transferred to other species. The only other species that we sampled from the Damariscotta River is rainbow smelt, *Osmerus mordax*; of 13 sampled in May 1975, 3 had characteristic PEN lesions.

Management practices that frequently involve transferring alewives from one stream to another for the development and maintenance of commercial runs (Rounsefell and Stringer 1943; Walton and Smith personal communication) have undoubtedly contributed to the spread of this disease and should be considered in the future management of this species. A case in point is the transfer, during the time of this study, of prespawning adults from the Damariscotta River fishery to Adams Pond in Boothbay for the initiation of a new spawning run. At the time of transfer, alewives from the Damariscotta fishery were exhibiting a 70% incidence of PEN.

Acknowledgments — I thank C. J. Walton and M. E. Smith (Maine Department of Marine Resources [MDMR]) for field assistance and advice on the local spawning habits of alewives; D. B. Sampson (MDMR) and K. A. Soucy (Bates College) for field assistance and Dr J. J. Graham (MDMR) who critically reviewed the manuscript. D. S. MacKenzie (Maine Medical Center) prepared the photomicrographs. J. A. Rollins (Bigelow Laboratories for Ocean Sciences) prepared Fig. 1.

APPY, R. G., AND M. D. B. BURT. 1975. Piscine erythrocytic necrosis (PEN) in the blood of the Atlantic cod (*Gadus morhua* L.). Can. Soc. Zool. Annu. Meet., Guelph, Ont. (Abstr.)

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Comparison of an Ecological Model of Lake Ontario and Phosphorus Loading Models[1]

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SCAVIA, D., AND S. C. CHAPRA. 1977. Comparison of an ecological model of Lake Ontario and phosphorus loading models. J. Fish. Res. Board Can. 34: 286–290.

Predicted responses of Lake Ontario to phosphorus loads from two empirical relationships and an ecological model were compared. Predictions of annual average concentrations of total phosphorus and chlorophyll *a* by the ecological model were consistent with those predicted by the simpler models. We concluded that the use of a particular type of model is governed by the nature of the problems being addressed rather than an inherent superiority of either approach.

SCAVIA, D., AND S. C. CHAPRA. 1977. Comparison of an ecological model of Lake Ontario and phosphorus loading models. J. Fish. Res. Board Can. 34: 286–290.

Nous comparons les réponses du lac Ontario à des charges de phosphore prédites à partir de deux relations empiriques et d'un modèle écologique. Les prédictions des concentrations moyennes annuelles du phosphore total et de la chlorophylle *a* dérivées du modèle écologique

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s'accordent avec celles dérivées des modèles plus simples. Nous en concluons que l'emploi d'un type de modèle particulier est dicté par la nature des problèmes étudiés plutôt que par une supériorité inhérente de l'une ou de l'autre approche.

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THE use of mathematical models of lake eutrophication in environmental management has been strongly endorsed by the U.S. Environmental Protection Agency (1971) and is the basic reason for most modeling programs. These models generally have developed along two lines: phosphorus load models and ecosystem models. The former use empirical correlations to relate lake trophic state to simple expressions of hydrology, morphometry, and phosphorus loading and are most notably represented by the loading plots of Vollenweider (1968, 1975) and Dillon and Rigler (1974a, b). These empirical models typically employ macroscopic parameters, such as mean annual concentration of total phosphorus or summer concentration of chlorophyll *a*, to assess trophic state. Ecosystem models (Park et al. 1974; Thomann et al. 1975; Lehman et al. 1975), on the other hand, represent a more mechanistic approach designed to simulate the seasonal dynamics of various components of lake biology and chemistry. Since both approaches address the same processes, albeit on different scales, their predictions should be consistent. If this can be shown to be true, then the selection of the type of model to be used for a specific application is more a matter of the scope of the problems to be addressed than of an inherent superiority of either approach. The primary purpose of the present note is to show, by comparing the output of an ecological model (i.e. an ecosystem model built upon ecologically sound process formulations) of Lake Ontario with predictions from some phosphorus loading models, that within certain limits the approaches agree reasonably well.

The ecological model — The ecological model was developed originally by Scavia et al. (1976a) for Lake Ontario and has been applied successfully to other Great Lakes (Scavia et al. 1976b). It has been modified and improved for the present analysis and calculates the dynamics of the following state variables on a daily basis:

Available P	5 groups of phytoplankton
NH_3	6 groups of zooplankton
NO_3	Inorganic C system
Dissolved organic N	Sedimentary C
Soluble reactive Si	Benthic macroinvertebrates
Particulate Si	Detrital C

The model treats the lake as homogeneous in the horizontal plane and segments it vertically into two layers representing epi- and hypolimnion. A physical model is used to calculate changes in segment thickness, temperature, and transport in response to winds and temperature at the air–water interface.

Each state variable in the ecological model is described by a differential equation composed of the sum of important biological and chemical processes affecting the concentration of that substance (e.g. photosynthesis, grazing, respiration, remineralization, excretion, defecation). Physical processes are also simulated. Sedimentation, diffusion, and gas exchange at the air–water interface are all computed within the model as functions of the seasonal environmental conditions (i.e. temperature, wind). External loadings of the state variables are considered as constant throughout the year and added, as a lake-wide average, to either the epilimnion or the whole lake, depending upon whether or not the lake is stratified. Additional details of the model's mathematical structure and the interrelationships of the above variables are presented elsewhere (Scavia et al. 1976a).

Comparison with phosphorus loading models — The ecological model is compared below with two models representative of the phosphorus loading approach. The first, developed by Vollenweider (1969) and modified by Dillon and Rigler (1974b) to predict mean annual concentration of total phosphorus for a lake at steady state, is formulated as follows:

$$p = \frac{L(1 - R_p)}{\rho z} \qquad (1)$$

where p is the mean annual concentration of total phosphorus (milligrams per cubic metre), ρ is the inverse of the water retention time (per year), z is the mean depth (metres), L is the areal load of total phosphorus to the lake (milligrams per square metre per year), and R_p is the phosphorus retention coefficient. Chapra (1975) suggested the following relationship to calculate R_p:

$$R_p = \frac{v}{v + q_s} \qquad (2)$$

where v is the apparent settling velocity of total phosphorus (metres per year), and q_s (outflow divided by lake surface area) is the areal water load (metres per year). Chapra (1975) evaluated v empirically, and his value of 16 m/yr is used in the following comparisons. Equations (1) and (2) can be combined to yield

$$p = \frac{L}{16 + q_s} \tag{3}$$

since $q_s = \rho z$.

The ecological model was run for 5–20 yr to reach steady state under a variety of loading conditions for Lake Ontario. Mean annual total phosphorus was determined by summing the annual integrated concentrations of available phosphorus and phytoplankton, zooplankton, and detrital phosphorus. The latter three were calculated by assuming a fixed stoichiometric composition of organic matter of 41:7.2:1 for C:N:P on a weight basis (Redfield et al. 1963). A similar ratio (35–51:7.7:1) can also be determined by examining the relative fractions of particulate organic carbon, nitrogen, and phosphorus in Lake Ontario (Stadelmann et al. 1974; Stadelmann and Fraser 1974).

A comparison of the models (Fig. 1) shows that the predictions of p are quite consistent. This is particularly interesting considering there are large differences between Lake Ontario and the lakes used to derive equation (3). For example, the mean depth of Lake Ontario is 86 m, whereas the mean depth of the lakes used to derive equation (3) is 10 m. The consistency is also significant when one considers the vast differences between the models. For example, equation (3) uses a constant settling velocity for total phosphorus, whereas the ecological model calculates individual sinking rates for detrital phosphorus and each phytoplankton group based on size, shape, and the seasonal variations in temperature and phytoplankton "vigor."

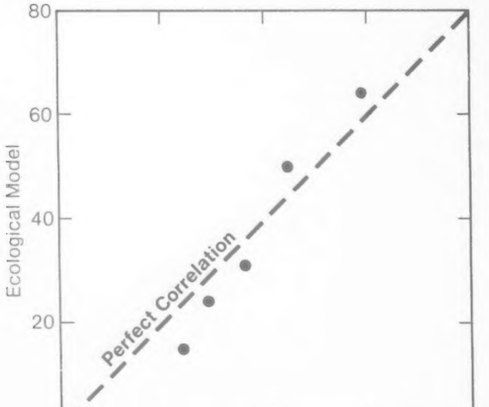

FIG. 1. Comparison of total phosphorus concentration (milligrams per cubic metre) as calculated by the ecological model and by equation (3).

The second comparison is between the ecological model and the following equation derived by Dillon and Rigler (1974a) relating the concentration of spring total phosphorus to that of summer chlorophyll a:

$$[\text{Chl } a] = 0.0731\,[P_v]^{1.449} \tag{4}$$

where [Chl a] is the mean epilimnetic concentration of chlorophyll a during summer (milligrams per cubic metre), and $[P_v]$ is the concentration of total phosphorus in the spring (milligrams per cubic metre). [Chl a] values are determined from the ecological model by integrating epilimnetic values of phytoplankton carbon (C:Chl $a = 50$) for the period from mid-May to the beginning of September (Dillon and Rigler's definition of summer). The ecological model was again run under a variety of loading conditions. While steady state values are not required for Dillon and Rigler's correlation, the spring total N:P ratio must be > 12. This is an empirically defined threshold below which equation (4) breaks down. Sakamoto (1966) hypothesized that this is due to nitrogen taking precedence as the primary limiting nutrient at ratios < 12. This hypothesis was supported when results from the ecological model simulations were compared with equation (4). For points representing N:P > 12, the correlation between the ecological model prediction and equation (4) is good ($r = 0.85$), although the scatter increases at higher values of phosphorus (Fig. 2). For points representing N:P < 12 (not on Fig. 2), however, the agreement deteriorates, with the chlorophyll values falling below Dillon and Rigler's line as would be expected if something other than phosphorus were limiting.

Since the ecological model is a mathematical representation of the proposed mechanisms operating within the lake, the limitations of its dynamics can be determined directly. For N:P > 12, as phosphorus levels increase, first phosphorus and then phosphorus and light are the predominant factors limiting phytoplankton growth. Nitrogen never approaches levels at which it significantly influences seasonal phytoplankton dynamics. For N:P < 12, however, nitrogen gains importance to the point where, at very low N:P levels, phosphorus is no longer the major control of algal productivity in the model. This may seem self-evident, but it suggests a possible use of

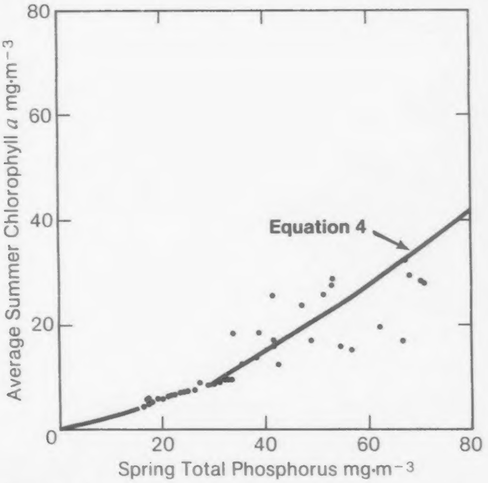

FIG. 2. Comparison of ecological model results (points) with Dillon & Rigler's (1974a) correlation (line) between average summer chlorophyll *a* and spring total phosphorus.

ecological models in elucidating the mechanisms of the more macroscopic modeling approaches.

When spring phosphorus concentrations are above approximately 75 mg/m^3, the two approaches diverge. While Dillon and Rigler's correlation continues to show an exponential relationship of chlorophyll *a* to phosphorus, the ecological model levels off, with nutrient limitation becoming subsidiary to light as the major factor governing lake productivity. A possible explanation is that the ecological model was developed for a large mesotrophic lake — Lake Ontario (spring phosphorus ~ 20 mg/m^3) — and certain mechanisms specific to hypereutrophic systems may not be included (e.g. heightened feedback of phosphorus from the sediments when the hypolimnion is anoxic). When Schindler (1976) compared two regression equations, developed from EPA-National Eutrophication Survey data and International Biological Program data, with equation (4) he observed a similar divergence at higher P concentrations. This anomaly should be investigated further.

At present the ecological model is being modified to include mechanisms describing more eutrophic processes and is being applied to several eutrophic lakes. When this is completed, the model will again be compared with phosphorus loading models to see if the modifications affect the consistency of the approaches for these hypereutrophic conditions.

The agreement between the model predictions indicates that model selection should be based upon the scope of the problems to be addressed. That is, if one is interested in the effects of phosphorus loadings on the annual average concentration of total phosphorus or chlorophyll, the loading concept approach should be used. These models have been designed for that purpose and provide quick, inexpensive estimates of the loading impact. If one is interested in investigating the effects of external loads on seasonal dynamics of various components of a lake ecosystem, interactions among these components, or the nature of the components themselves, an ecological model should be used. These models generally represent mathematical aggregates of a number of aquatic biological and chemical processes and can provide more detailed predictions of the effects that will result.

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Factors in Interpreting Data Obtained by Diel Sampling of Fish Stomachs[1]

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Eggers, D. M. 1977. Factors in interpreting data obtained by diel sampling of fish stomachs. J. Fish. Res. Board Can. 34: 290–294.

A simple differential equation model describing the level of food in the stomach, incorporating simultaneous ingestion and gastric evacuation, is used to gauge the sensitivity of methods that utilize diel change in food level of the stomach to determine diel feeding activity of fish. Analysis of model behavior under different feeding regimes shows that diel pattern of food level in the stomach is less sensitive to feeding regime than many authors claim.

An expression is derived for the magnitude of bias associated with cessation of feeding and continuance of gastric evacuation while fish are retained by the fishing gear. This bias may be reduced by minimizing sampling time and accelerating preservation of captured fish in experiments to determine feeding chronology and daily ration.

Eggers, D. M. 1977. Factors in interpreting data obtained by diel sampling of fish stomachs. J. Fish. Res. Board Can. 34: 290–294.

Dans le but d'évaluer la sensibilité de méthodes utilisant le changement nycthéméral de la quantité de nourriture dans l'estomac pour déterminer le cycle alimentaire journalier des poissons, l'auteur se sert d'une simple équation différentielle décrivant le niveau de nourriture dans l'estomac et incorporant l'ingestion et l'évacuation gastrique simultanées. L'analyse du comportement du modèle sous différents régimes d'alimentation démontre que le cycle journalier du niveau de nourriture dans l'estomac est moins sensible au régime d'alimentation que ne le soutiennent plusieurs auteurs.

On obtient du modèle une expression de la grandeur du biais associé à l'arrêt de l'alimentation et à la continuation de l'évacuation gastrique pendant que les poissons sont retenus par l'engin de pêche. Un moyen de diminuer ce biais est de réduire au minimum le temps d'échantillonnage et de préserver le plus rapidement possible les poissons capturés lors d'expériences visant à déterminer la chronologie alimentaire et la ration quotidienne.

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It is a common practice to determine the diel timing of feeding for fish populations by observing daily variation in the level of food in the stomach (Darnell and Meierotto 1962; Girsa 1975; Gjösaeter 1973; Holton 1969; Merrett and Roe 1974; Narver 1970; Swenson and Smith 1973). The level of food in the stomach varies as a function of the rates of food ingestion and gastric evacuation. As these rates are simultaneous and interdependent, accurate description of diel variation in rate of food ingestion by the aforementioned methodology depends on consideration

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of both rates. Furthermore, feeding usually ceases in captured fish, while gastric evacuation continues. Therefore, the estimated level of food in the stomach of the sample fish is lower than that for the whole population. The magnitude of this bias in estimating food level in the stomach is investigated, and an attempt is made to correct for it.

Results and discussion — A simple differential equation model incorporating ingestion and gastric evacuation was used to predict the time trajectory of the food level in the stomach under different diel feeding regimes. These trajectories were evaluated with respect to improving methodology on feeding chronology.

The level of food in the stomach at any time can be determined from

$$\frac{dS}{dt} = R - \alpha S \qquad (1)$$

where S is the amount of food in the stomach, t is time, R is the rate of food intake, α is the rate of gastric evacuation. Equation (1) assumes that gastric evacuation is an exponential decay function (Elliott 1972; Edwards 1971; Windell 1966; Brett and Higgs 1970; Molnár and Tölg 1962).

Four hypothetical formulations of the daily feeding regime were considered.

Case I: R is constant during a 12-h period, then zero for 12 h. This case represents a visual predatory fish feeding continuously at constant rate during daylight only.

Case II: R linearly increases during a 12-h feeding period with R being zero at other times of the day.

Case III: R is constant during two 2-h feeding periods. One follows sunrise, the other precedes sunset. R is zero at other times of the day. Similar feeding regime was observed in juvenile sockeye salmon (*Oncorhynchus nerka*) by Narver (1970) and in black bullheads (*Ictalurus melas*) by Darnell and Meierotto (1962).

Case IV: R is constant during a 4-h feeding period preceding sunset and R is zero at other times of the day.

The instantaneous rate of food intake for these four feeding regimes is shown in Fig. 1. Note that in each case the total daily food intake is constant (14.4 mg dry wt/day).

The time trajectories for the level of food in the stomach can be determined by solving equation (1) under each feeding regime. The analytical solution of the differential equation is the same for feeding regime cases I, III, and IV, but different for case II.

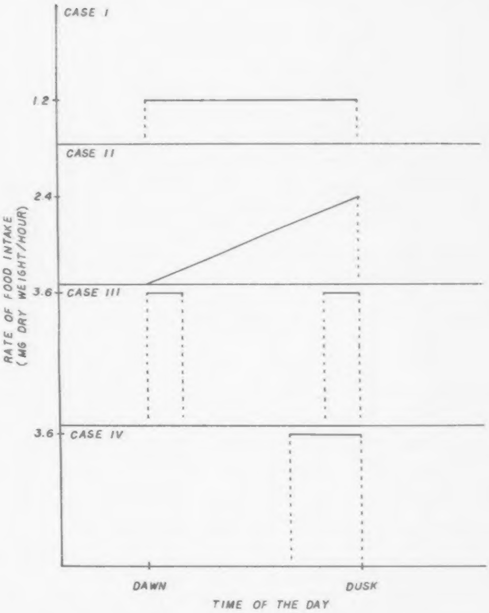

FIG. 1. Instantaneous rate of food intake (R) under four diel feeding regimes.

The equation for cases I, III, and IV can be solved by separating variables and integrating. The solution is:

$$\text{If } R > 0, \quad S = \frac{R - \exp[-\alpha(t + C_1)]}{\alpha} \qquad (2)$$

$$\text{If } R = 0, \quad S = C_2 \exp(-\alpha t) \qquad (3)$$

Note that equation (3) describes the well-known exponential decay of stomach contents observed in nonfeeding fishes.

The equation for case II is a first order linear differential equation and can be easily solved:

$$\text{If } R > 0, \quad S = \frac{R(\alpha t - 1)}{\alpha^2} + C_3 \exp(-\alpha t) \qquad (4)$$

If $R = 0$, the solution is equation (3).

In the above equations, C_1, C_2, and C_3 are constants that can be determined from initial conditions.

The level of food in the stomach at the end of feeding provides initial conditions for the interval where equation (3) operates. The level of food in the stomach at the end of the nonfeeding period provides the initial conditions for the interval where equation (2) or equation (4), depending on the feeding regime, operates. Note that C_2 is in units of milligrams dry weight but units of C_1 and C_3 are more complicated.

Solutions of equation (1) for each feeding regime are given (Fig. 2), where $S(t) = S(t + 24)$ and the assumed gastric evacuation rate $(\alpha) = 0.2\ h^{-1}$. The results suggest that differences in the hypothetical feeding regimes represented by cases I, II, and IV would be difficult to detect with limited data. This difficulty would be compounded by vertical gradients of prey abundance, species composition, light intensity, etc., that contribute to variability in food level in the stomachs of individual fish. From the data presented by Gjösaeter (1973), Merrett and Roe (1974), Girsa (1975), one can only infer that fish are feeding during the day.

If fish are feeding during brief but intense periods separated by a time interval sufficient to change substantially the level of food in the stomach during that interval (case III), then diel sampling will successfully detect this type of feeding chronology (Narver 1970; Darnell and Meierotto 1962). Techniques that consider the freshness or degree of digestion of individual prey items (Swenson and Smith 1973) supply more information and should be used to complement diel sampling to determine feeding chronology of fish populations.

It is possible to estimate the daily ration from diel trajectories of the level of food in the stomach. Bajkov (1935) and Darnell and Meierotto (1962) propose the following formula:

$$R_T = 24\bar{S}\alpha \qquad (7)$$

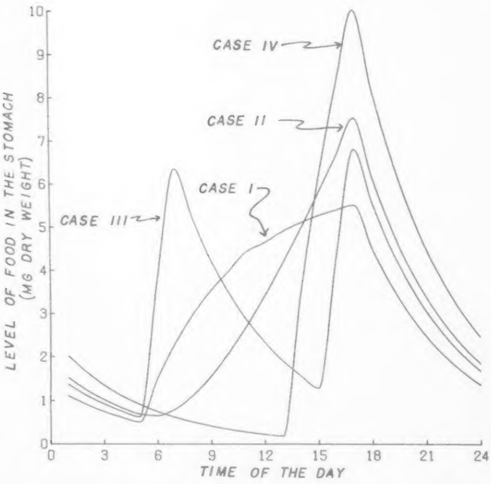

FIG. 2. Diel trajectories of the level of food in the stomach under the four diel feeding regimes.

where R_T is daily ration, and $\bar{S}$ is the average level of food in the stomach during the 24-h period.

There are two common sources of bias that affect estimation of $\bar{S}$. The first stems from insufficient sampling to identify true minimum or maximum levels of food in the stomach. This problem is more serious in fish populations that feed intensely during brief time periods. The solution is more frequent sampling. The second source of bias is extended retention time, for while fish are retained in the gear, ration intake is usually zero but gastric evacuation continues. The actual bias can be computed from the solutions to equation (1), assuming that fish are being caught in the gear at a constant rate.

To derive the expression for the second source of bias, the following must be considered. Let t_s be the start of fishing and t_e be the end of fishing. The time interval of sampling is $T = t_e - t_s$. Let $S(t)$ be the level of food in the stomach for the population at time t. Note that $S(t)$ is given by the solution to equation (1). Let $G(t)$ be the estimated level of food at t_e in the stomach for an individual fish captured in the gear at time t. Note that $t_s \leqslant t \leqslant t_e$.

$G(t)$ is given by the level of food in the stomach at the time the fish is caught, $S(t)$, times the decrease in the level of food in the stomach due to gastric evacuation while the fish is retained by the gear. That is,

$$G(t) = S(t)\exp[-\alpha(t_e - t)] \qquad (8)$$

Let $\bar{G}$ be mean level of food in the stomachs estimated from all of the fish in the sample. Thus,

$$\bar{G} = \frac{\int_{t_s}^{t_e} G(t)dt}{T} \qquad (9)$$

Now we can determine the magnitude of bias B:

$$B = \frac{\bar{G} - S(t_e)}{S(t_e)} \qquad (10)$$

If R is constant (i.e. cases I, III, or IV), it is easy to solve equation (9) for the analytical expression for $\bar{G}$, hence B. As the level of food in the stomach at the initiation of sampling increases, B decreases (Fig. 3). Here, the parameter values used to derive B are identical to those of case I, and T is equal to 1 h. Clearly the magnitude of bias is large. B reaches a minimum at the steady state conditions of equation (1), i.e. $S = R/\alpha$. This situation is not likely to occur naturally because either R declines as satiation approaches (Ivlev 1961; Ware 1972) or feeding

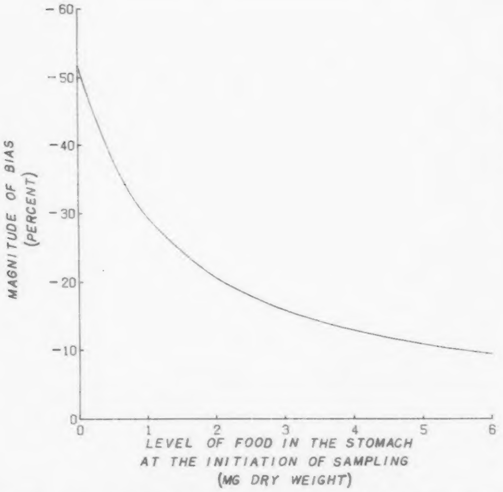

FIG. 3. Relation between the magnitude of bias and level of food in the stomach at the initiation of sampling. The equilibrium level of food in the stomach is 6 mg dry wt.

ceases with onset of darkness. However, it is useful to consider the bias for the steady state situation because it constitutes the lower limit on the actual bias, and at steady state conditions equation (9) is greatly simplified.

At steady state the level of food in the stomach is constant. Thus,

$$\bar{G} = \frac{S^*[1 - \exp(-\alpha T)]}{\alpha} \qquad (11)$$

$$B = \{(1/\alpha)[1 - \exp(-\alpha T)]\} - \alpha \qquad (12)$$

where S^* is the steady state level of food in the stomach. Thus, the steady state bias is purely a function of α and T. The magnitude of bias (B) increases with duration of sampling and with rate of gastric evacuation (Fig. 4). If the stomach is in steady state, it is easy to adjust the estimated level of food in the stomach to the population food level in the stomach if one knows the rate of gastric evacuation. From equation (11)

$$S^* = \frac{\alpha\bar{G}}{1 - \exp(-\alpha T)} \qquad (13)$$

Kapoor et al. (1975) state that gastric evacuation depends among other things on the rate of secretion of gastric juices and gastric peristalsis. Both processes cease with death. Therefore, the rate of gastric evacuation for the fish that are killed by the sampling gear is less than that in the population. The actual bias is less than the theoretically derived bias if the fish are killed in the gear. However, since gastric digestion is a chemical process, the quantity and quality of the food in the stomach decreases after death of the fish.

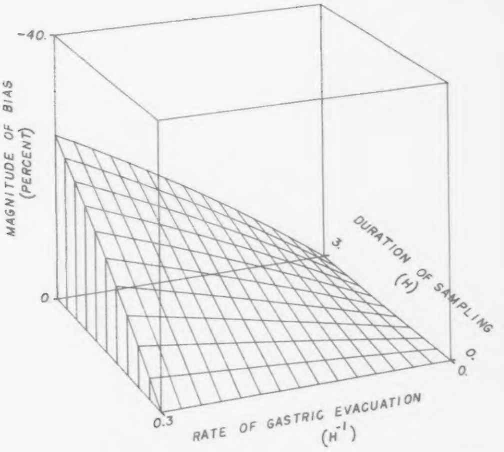

FIG. 4. Response of the magnitude of bias to gastric evacuation rate and duration of sampling.

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Sex Ratio of American Eels, *Anguilla rostrata*, from the Matamek River System, Quebec, with Remarks on Problems in Sexual Identification[1]

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DOLAN, J. A., AND G. POWER. 1976. Sex ratio of American eels, *Anguilla rostrata*, from the Matamek River system, Quebec, with remarks on problems in sexual identification. J. Fish. Res. Board Can. 34: 294–299.

The failure to examine gonadal tissue of American eels, *Anguilla rostrata*, histologically could result in considerable errors in sexual identification. Two "organs of Syrski", usually considered to be the male testes, and several small, ribbon-like gonads, generally thought to indicate female ovaries, revealed contradictory results when sectioned histologically. Other sources of error in determining a representative sex ratio of eels were noted. In the Matamek River system, Quebec, the range in length at which gonadal formation commenced was considerable. Male eels were rare (5 of 613 fish), which is consistent with other reports for the northern limits of this species.

DOLAN, J. A., AND G. POWER. 1977. Sex radio of American eels, *Anguilla rostrata*, from the Matamek River system, Quebec, with remarks on problems in sexual identification. J. Fish. Res. Board Can. 34: 294–299.

On peut faire beaucoup d'erreurs dans la détermination du sexe de l'anguille d'Amérique si l'on ne fait pas l'examen histologique du tissu de la gonade. Deux «organes de Syrski», ordinairement considérés comme étant les testicules du mâle, et plusieurs petites gonades en forme de ruban, généralement prises pour les ovaires de la femelle, donnent des résultats contradictoires lorsque sectionnés histologiquement. Les auteurs notent d'autres sources d'erreur dans la détermination d'une proportion représentative des sexes de l'anguille. Dans le réseau de la rivière Matamek, Québec, la gamme de longueurs auxquelles commence la formation des gonades est très étendue. Les anguilles mâles sont rares (5 sur 613 poissons), ce qui est en accord avec d'autres observations aux limites septentrionales de cette espèce.

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IT has been stated that male American eels, *Anguilla rostrata*, are found principally in estuarine or coastal marine habitats while females are confined mainly to fresh water (Bigelow and Schroeder 1953; Vladykov 1966; Böetius and Böetius 1967). However, a review of the literature reveals no evidence to confirm this distribution of sexes (see Table 1). Moreover, Winn et al. (1975) demonstrated exactly the opposite distri-

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TABLE 1. Comparison of the sex ratios of American eels, *Anguilla rostrata*, from various localities (arranged in approximately decreasing latitude). Unavailable information is indicated by a dash.

				Length range (*cm*)			
	Habitat[a]	Gear[b]	% Male	Males	Females	Total no. examined	Source
Newfoundland	F	E,F,S	1	25.2	22.7–93.1	184	Gray and Andrews (1970)
Newfoundland	M	P	0		28.1–77.0	154	Gray and Andrews (1970)
Newfoundland	—	—	6	19.3–34.3	26.8–93.0	191	Vladykov (1966)
Quebec	—	—	1	25.5	19.3–127.5	164	Vladykov (1966)
Ontario	F	—	0		42.2–103.0	151	Vladykov (1966)
Nova Scotia	—	—	0		20.3–73.2	37	Vladykov (1966)
New Brunswick	—	—	20	21.2–37.5	22.1–64.2	198	Vladykov (1966)
Rhode Island	F	F,W	88	—[c]	—[c]	1413	Winn et al. (1975)
Rhode Island	M	F	55	—[c]	—[c]	106	Winn et al. (1975)
Massachusetts	—	—	9	26.2–33.3	25.8–41.5	107	Vladykov (1966)
New York	—	—	33	22.5–35.7	24.0–62.5	106	Vladykov (1966)
—[d]	F	E,P,T,L	11	—	—	259	Huver (1966)
—[d]	M	E,P,T,L	0		—	124	Huver (1966)
Virginia	—	—	0		40.0–64.2	41	Vladykov (1966)
Virginia[e]	—	Po	53	33.9–43.8	35.2–68.7	98	Wenner and Musick (1974)
Louisiana	—	—	83	20.6–29.5	44.0	6	Vladykov (1966)
Florida	—	—	53	20.6–37.5	22.9–59.2	36	Vladykov (1966)
Bermuda	M	M	4	29.5–32.7	17.8–57.6	88	Boëtius and Boëtius (1967)
Trinidad	—	—	38	26.9–32.7	31.4–49.6	16	Vladykov (1966)

[a] F — fresh water, M — marine or brackish water.

[b] E — electrofishing, F — fyke net, S - smolt trap, P — eel pot, W — Wolf-type trap, T — trapnet, L — longline, Po — Poundnet, M — minnow trap.

[c] Length ranges for silver eels were given as 28–40 cm (male) and 41–80 cm (female) but the authors failed to clarify whether sex ratio data were based only on silver eels.

[d] Samples from United States, but exact location not given.

[e] These were silver eels obtained from commercial fishermen and are not representative of the sex ratio of eels from this region.

bution of males and females in Rhode Island waters. Researchers probably assumed that the distribution would be similar to that postulated by Bertin (1956) for the closely related European eel, *Anguilla anguilla*. Yet Bertin himself cited numerous exceptions to the "female-freshwater, male-saltwater" theory. Recently Sinha and Jones (1966) studied the sex ratio of 8000 European eels from Welsh rivers and concluded that "contrary to popular belief, males and females were found together in fresh water and brackish water."

Male American eels are rare in the northern parts of their range (Vladykov 1966; Gray and Andrews 1970). Vladykov (1966) claimed that there was a north–south gradient of sex ratio along the Atlantic coast of North America (see Table 1) but the failure to demonstrate a random, nonselective sample (Winn et al. 1975) as well as the small number of specimens examined from some localities renders his conclusions doubtful.

Mature male eels are usually smaller than mature females (Vladykov 1966) and this criteria was used by Winn et al. (1975) to sex some of their Rhode Island silver eels. The sex of the remaining eels was determined by gross morphological examination of the gonads, a technique also used by Vladykov (1966) and Böetius and Böetius (1967). Huver (1966), Gray and Andrews (1970), and Wenner and Musick (1974) all employed histological methods to confirm sexual identification.

This paper examines the sex ratio of American eels from various parts of the Matamek River system, Quebec. During the course of investigations several problems with the morphological identification of male and small female eels were encountered. An attempt is made to standardize the methods used by researchers on the sex of eels to avoid incorrect identifications and render results more comparable between studies.

Materials and methods — The Matamek River is situated on the north shore of the Gulf of St. Lawrence approximately 32 km east of Sept Iles, Quebec. Further description of the watershed may be found in Gibson (1973) and Power et al. (1973). Bill Lake has been described by O'Connor and Power (1973). Ruisseau Sylvie is a small stream (length, 2.8 km; average width, 6 m) about 3.2 km east of the mouth of the Matamek River.

Eels were caught during the summers of 1973–75, mainly in fyke nets (0.6 cm^2 mesh) and on longlines, but eel traps, minnow traps, and rotenone (Ruisseau Sylvie only) were also employed. Fish were anesthetized with MS-222® shortly after capture. Total length from the tip of the lower jaw to the posterior end of the middle caudal ray was measured to the nearest millimetre. Gonadal tissue, when present, was examined and subsequently fixed in Bouin's solution for 48 h, then washed and stored in 70% ethanol. Histological sections were prepared by standard techniques (Mahoney 1973). Paraffin sections were cut at 10 μm on either a "Cambridge Rocker" microtome or a rotary microtome, mounted on slides, stained with hematoxylin, counterstained with eosin, and observed under a compound microscope.

Results — Histologically, female eels were identified by the presence of developing oocytes and males by the presence of developing spermatogonia (Gray and Andrews 1970; Wenner and Musick 1974) (Fig. 1 and 2a, b).

Morphologically, all male eels possessed the "organ of Syrski" (Bertin 1956) or lobed gonadal tissue (Fig. 2c). The external appearance of the female gonad resembled a frilled ribbon-like structure (Bertin 1956). The gonads of smaller female eels (usually <30 cm) were generally small ribbon-like structures with few crenulations. A number of eels with such gonadal tissue contained only undifferentiated tissue when examined histologically. Of two additional eels with "organs of Syrski", one was identified histologically as a female (35.3 cm, Fig. 2d) and the other contained only undifferentiated tissue (25.7 cm).

The length at which gonadal development commenced was extremely variable. Table 2 presents the change with length in the relative proportion of immature eels (eels with undifferentiated tissue or no gonadal tissue present) to eels that could be sexed (eels with differentiated tissue). The longest immature eel was 37.5 cm.

A total of 613 eels could be sexed from the Matamek River system, ranging in length from 16.6 cm to 87.0 cm (Table 3). Only five of these were male — three from the Matamek River (fresh water), with lengths of 34.6, 39.6, and 50.3 cm, and two from Ruisseau Sylvie (brackish water) whose lengths were 27.0 and 33.6 cm.

TABLE 2. Change with length in the relative proportion of immature eels to eels that could be sexed.

Range length (*cm*)	% sexed	% immature	No. examined
<15.0	0	100	200
15.0–19.9	8	92	74
20.0–24.9	43	58	94
25.0–29.9	85	15	97
30.0–34.9	93	7	96
35.0–39.9	99	1	96
≥40.0	100	0	301

TABLE 3. The sex of eels from various parts of the Matamek River system.

Location	Length	Male	Female	Total
Matamek River (fresh water)	16.6–82.5	3	549	552
Matamek River (brackish water)	20.9–58.7	0	30	30
Ruisseau Sylvie (brackish water)	17.0–39.2[a]	2	10	12
Bill Lake (fresh water)	33.2–87.0	0	19	19
Totals		5	608	613

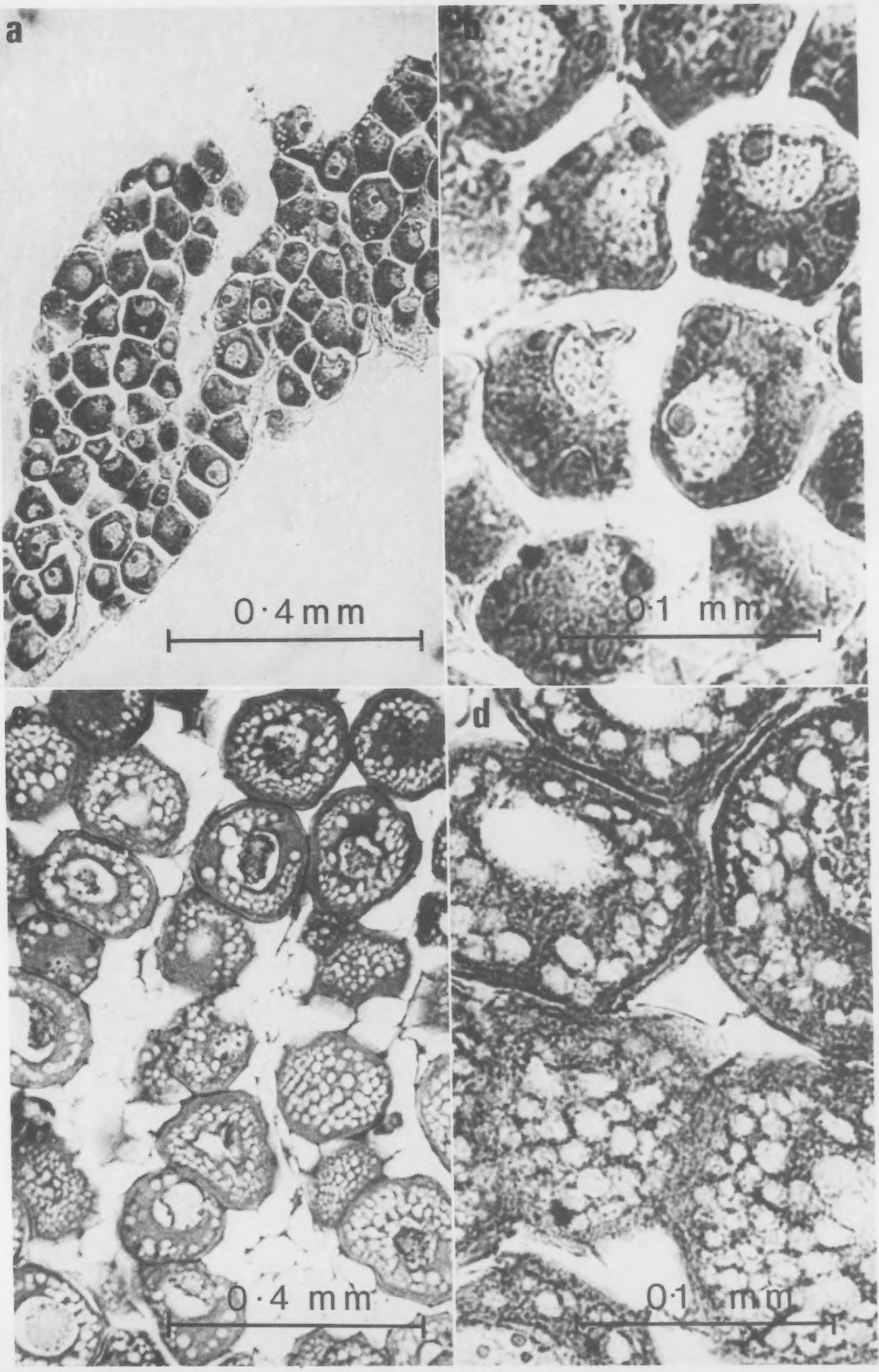
[a]Lengths measured after preservation in 70% ethanol.

Discussion — The apparent ambiguity of the "organ of Syrski" as an indicator of maleness in the American eel has also been demonstrated in the European eel (Sinha and Jones 1966). The appearance in our study of "female-like" gonads that were undifferentiated histologically introduces further evidence that studies employing macroscopic examination to determine the sex of American eels (Vladykov 1966; Böetius and Böetius 1967) could contain erroneous results. We recommend that all eels containing very small ribbon-like gonads or those possessing the "organ of Syrski" should be examined histologically to confirm sexual identification.

The range in length at which gonadal formation begins is consistent with the variable and

FIG. 1. a, b, Transverse section (TS) of female gonadal tissue; total length of specimen = 28.1 cm; c, d, TS of female gonodal tissue; total length of specimen = 59.4 cm.

FIG. 2. a, b, TS of male gonadal tissue; total length of specimen = 33.6 cm. c, external appearance of silver male eel gonads ("organ of Syrski"); total length of specimen = 50.3 cm; note overlapping of lobes in this large specimen; d, photograph of "organ of Syrski" that was histologically female; total length of specimen = 35.3 cm.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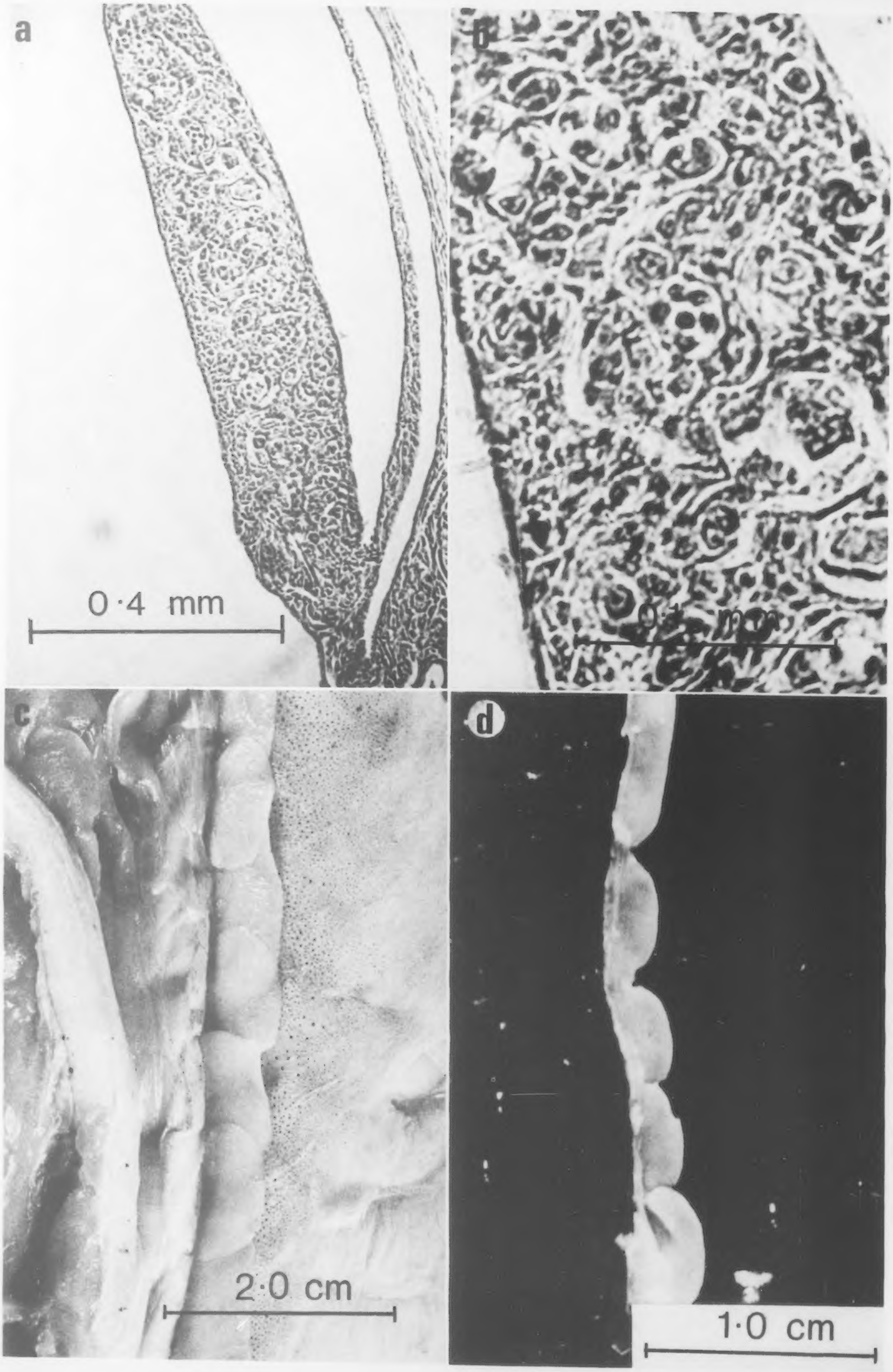
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unpredictable growth characteristics of the species in this river (Dolan 1975). Gray and Andrews (1970) found immature eels as long as 33.6 cm. That most eels <20 cm in length are immature is consistent with the findings of Vladykov (1966) and Gray and Andrews (1970).

Vladykov (1966) stated that male American eels did not grow as large as male European eels, the latter reaching a maximum length of 50.8 cm. Our 50.3 cm specimen (Fig. 2c) is larger than any male American eel recorded in the literature and is similar in length to the maximum size for the European species. Since mature male eels may reach a considerable size, a separation of the sexes by length alone as done in part by Winn et al. (1975) could underestimate the number of males in the sample.

The small number of males in our Matamek River samples is consistent with the results of Vladykov (1966) and Gray and Andrews (1970) (see Table 1). However, little can be concluded on the distribution of the sexes in salt and fresh water as our brackish water samples are rather small. The variety of relatively nonselective methods used to capture eels, the continuous seasonal sampling, and the use of histological techniques to identify sex probably produced a true picture of the sex ratio of eels in the fresh waters of the Matamek River system. Factors such as differences in behavior or environmental preferences between the sexes (Gray and Andrews 1970) should not be totally discounted.

The reasons for the controversy over the geographical distribution of sex in the American eel could be a result of incorrect sexing, selectivity of the sampling gear and hence the possible exclusion of smaller male eels, and the a priori assumption that differences in habitat would be similar to those postulated by early European workers.

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Fish Orientation Behavior: An Electronic Device for Studying Simultaneous Responses to Two Variables

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REYNOLDS, W. W. 1977. Fish orientation behavior: an electronic device for studying simultaneous responses to two variables. J. Fish. Res. Board Can. 34: 300–304.

An electronic device ("Ichthyotron") is described that permits a fish to simultaneously and independently control (behaviorally regulate) two environmental variables by its movements along two axes, among four chambers. Sample data are given for simultaneous behavioral thermoregulation, light intensity preferences, and locomotor activity rhythms in largemouth bass, (*Micropterus salmoides*) and smallmouth bass (*M. dolomieui*).

REYNOLDS, W. W. 1977. Fish orientation behavior: an electronic device for studying simultaneous responses to two variables. J. Fish. Res. Board Can. 34: 300–304.

Nous décrivons un appareil électronique («ichtyotron») permettant à un poisson de contrôler simultanément et indépendamment (par comportement) deux variables de l'environnement par ses mouvements suivant deux axes, dans quatre chambres. A titre d'exemple, nous présentons des données sur la thermorégulation par comportement simultané, sur les préférences d'intensité lumineuse et sur les rythmes d'activité locomotrice chez l'achigan à grande bouche (*Micropterus salmoides*) et l'achigan à petite bouche (*M. dolomieui*).

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FISHES and other mobile organisms, by their movements, can exert considerable control over the environmental conditions they experience. Such habitat selection or orientation behavior has been studied in field and laboratory by a number of means, usually involving visual observations of the relative amounts of time spent by an animal in various available environmental conditions, as in a choice-chamber apparatus. Where the orientation behavior directly affects the physiology of the organism as an ultimate factor, the term "behavioral regulation" applies, e.g. behavioral thermoregulation. In its broadest sense, the term "orientation behavior" can be extended to the temporal as well as the spatial organization of an animal's activity, as in the case of locomotor activity rhythms.

Fry (1958) has reviewed various experimental gradient and choice-chamber designs going back to 1913 (Shelford and Allee), involving visual observation of the fishes' movements. Rozin and Mayer (1961) pioneered a new approach by allowing a fish to control water temperature by pressing a lever. Van Sommers (1962) employed a device in which fish controlled oxygen content of water by interrupting a photocell light beam. Kleerekoper (1967) used several different experimental chambers employing photocells to monitor fish movements. Various shuttlebox devices have been used by psychologists for many years to study avoidance conditioning, and these have also been used to study thermoregulation in lizards (Cabanac and Hammel 1971; Hammel et al. 1973) and fishes (Crawshaw and Hammel 1973). A shuttlebox device using photocells to monitor fish movements and control temperatures was described by Frank and Meyer (1971, 1974). Finally, Neill et al. (1972) described an improved electronic shuttlebox device for fish thermoregulation and suggested other possibilities for its use.

Responses of fishes to simultaneous opposed gradients of temperature and light in a single-axis gradient trough have been studied (DeVlaming 1971; Reynolds et al. 1977). For the study of simultaneous and independent responses to two variables such as light and temperature, a two-axis device is necessary. An early design (16 chambers, 4 × 4) requiring visual monitoring has been described (Reynolds 1973; Reynolds and Thomson 1974). The present communication describes a four-chamber, two-axis, electronically controlled device (the Ichthyotron) for crossed-gradient studies, with automatic data acquisition. This is the first full description of this device, with sources and costs of components, and sample two-factor regulation data.

Construction and Use — A schematic diagram of the arrangement of components in the device

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is shown in Fig. 1. The major electronic components (from Microswitch Division of Honeywell, Freeport, Ill. 61032) include TLS-3 adjustable focus light sources for detecting small objects ($9.90[1] each), TPC-O4F fast-response phototransistors ($18.50 each), and G4B control base with DPDT relay ($60 each) and LOG 3 logic function cards ($20 each) with immediate on–off response to light or dark. Total cost for eight each of the above was $867.20. Two Potter and Brumfield (Princeton, IN 47670 USA) 4PDT latching relays #KB17AG ($13.75 each) were also used. Esterline-Angus (POB 24000, Indianapolis, Ind. 46224) Minigraph Temperature ($195 each) and Event ($150 each) recorders were used; the temperature recorders also have event pens, making them dual-purpose.

The tank itself is constructed of plywood sealed with epoxy glue, painted with waterproof white epoxy paint. Silicone aquarium sealer was used for final waterproofing of joints. Glass windows permit passage of light beams for the phototransistors. Overall dimensions are 120 × 120 × 46 cm. Adjustable, sectioned, transparent plexiglass gates slide vertically in tracks between the light beams in the constrictions between chambers. These permit the openings between chambers to be adjusted to the size of the test animal, so that it can pass easily without missing the paired light beams. The openings can be at the bottom, middle or top of the water column in accordance with the habits of the species; the light sources and phototransistors are adjusted accordingly. By closing two of the gates, a two-chamber, single-axis configuration can be used to study responses to a single variable.

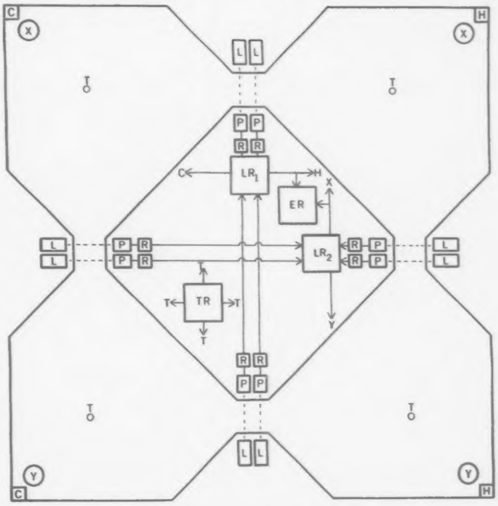

FIG. 1. Schematic diagram of the Ichthyotron (top view; 120 × 120 × 46 cm). L = light sources; dotted lines = light beams; P = phototransistors; R = phototransistor relays; LR = latching relays; C = cooling equipment; H = heaters; ER = event recorder (two channels); TR = temperature recorder (four channels); T = thermistors; X, Y = other variable(s) controlled by LR$_2$. As the fish moves between chambers, it sequentially interrupts two light beams, triggering phototransistor relays. The last relay activated determines the state of a bistable latching relay, which in turn controls the desired variable and activates the event recorder. The separate latching relays for each axis permit two variables to be controlled simultaneously and independently. The event recorder provides a continuous, unambiguous record of the animal's movements.

Immersion-type aquarium heaters are used for heating, and cooling is provided by pumping refrigerated water through Tygon tubing. The water in each chamber is well mixed and aerated with airstones, preventing thermal lamination. Temperature is varied by a simple "on–off" control and will continue to increase so long as the heaters are on in the "hot" chambers or to decrease so long as the refrigeration pumps are on for the "cold" chambers. Thus, the temperatures of the "hot-side" and "cold-side" chambers are continually either increasing or decreasing in parallel, and the differential between the two is controlled by thermal lag in heat transfer through the constrictions between the hot and cold sides. While the differential between chambers can be controlled more precisely by a comparator or some other arrangement (e.g. Neill et al. 1972), and the relative rates of heating and cooling can be varied (Beitinger 1976), these variables have been shown not to affect thermoregulatory performance of fishes, i.e. the fishes make appropriate adjustments in their shuttling behavior (Beitinger 1976). Therefore, these variables need not be precisely controlled. In fact, fishes will even learn to thermoregulate in such a device with no difference in temperature between the two chambers (a purely "temporal" gradient), but the instantaneous small spatial gradient of about 2°C caused by thermal lag helps to guide the fish to cooler water if it is too hot (thereby initiating further temporal cooling), or vice versa if it is too cold. This minimizes the learning time necessary for the fish to orient in the device, and species used to date have generally learned to use the device quickly (usually in <1 h).

At any one time, there are two different temperatures available in the device, the "hot-side" and the "cold-side" temperatures, usually differing by about 2°C. The mean of these two temperatures is the mean water temperature. The fish shuttles back and forth between these, but at any instant occupies either one or the other (the "occupied temperature"). The body temperature of the fish will generally lie between the two available water temperatures if it is shuttling rapidly, but will approach the occupied temperature if it remains in one chamber for long, depending on the size of the fish and its thermal equilibration rate in relation to the rate of change in the dynamic water temperature. The gut temperature of the fish can also be measured directly by telemetry, using a small ingestible temperature transmitter (Mini-Mitter Co., POB 88210-G, Indianapolis, Ind. 46208). While fishes have been shown to heat and cool at somewhat unequal rates due to temperature-dependent changes in heartbeat and ventilatory frequencies (Reynolds 1976, 1977), mean gut temperatures of fish over a period of time in this device do not differ significantly from mean occupied temperature or from mean water temperature over the same time period (Reynolds et al. 1976). Mean occupied temperature, determined with event and temperature records, takes into account the positions, and therefore, temperatures (there is a continuous record of both) occupied by the fish over time. Mean water temperature simply involves averaging the water temperature records of the hot and cold sides over the desired time period. These have been found not to differ significantly. Thus, mean occupied, water, and body temperatures over a period of time may be taken as essentially equivalent in this system. Occupied temperature is the variable most frequently used and is equivalent to preferred temperature. Statistics used to present the results can include mean, median, mode, range, midpoint, skewness, and kurtosis; the entire relative frequency distribution histogram may be shown (for a complete review of the relations among these measurements, see Reynolds and Casterlin 1976a).

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[1] Prices quoted for 1974.

The thermoregulatory system described above constitutes use of the circuitry in the "active" mode, essentially acting as an extended effector system of the fish. Any variable that can be controlled electrically or mechanically can be used in this way, i.e. heaters, refrigeration units, motors, pumps, solenoids, relays, rheostats, or valves can be used as the "effectors," and the only limitation on possible uses is one's imagination. Control can be "on–off" or variable. Rates of change and relative values in each chamber can be manipulated in various ways. Separate control systems can be used for each axis, as each has independent circuitry.

The device can also be used in a "passive" mode, in the manner of a classical choice chamber or shuttlebox, with automatic data acquisition through the photocell circuitry to monitor the movements of the test animal. In this mode, alternative fixed conditions are presented in each chamber. One axis can be used in the active mode, while the other is simultaneously used in the passive mode; for example, a fish can thermoregulate in the manner described above along one axis, with a choice of fixed light intensities along the other. Switching the light axis to the active mode, the fish can also turn the lights on or off by its movements along this axis. In any of the above modes or combinations of modes, the continuous activity record for each axis provides information that can be used to study locomotor activity rhythms. A timer can be used to provide any desired exogenous cycles, or the animal can be allowed to "free-run" under constant or self-controlled conditions.

Many other kinds of studies are possible with devices such as this one. The flexibility and automatic data-acquisition features of shuttlebox devices with electronic control and monitoring are certain to greatly facilitate future laboratory studies in orientation behavior. Fishes tested to date in the Ichthyotron include various centrarchids, ictalurids, catostomids, cyprinids, and esocids. Frog tadpoles (Reynolds and Covert 1976) and crayfish have also been successfully tested.

Examples of two-factor regulation by basses — Simultaneous responses to light and temperature were studied in largemouth bass (*Micropterus salmoides*) and smallmouth bass (*M. dolomieui*) (Fig. 2). With an exogenous 12 h L:12 h D light cycle, occupied-temperature distributions differed between night and day (Fig. 2A) for both species, an indication of thermoperiodism. Kurtosis of the distributions was greater during the "day" periods than during the "night" periods, and skewness also shifted between night and day.

During the "day" period of the light cycle bass were given a (passive-mode) choice between intensities of 1000 lx and 10 lx on the "light" axis. Largemouth bass spent much more time on the bright (1000 lx) side than did the smallmouth bass (Fig. 2B). At night the illumination in all chambers was about 1 lx, and the difference between species in proportions of time spent on each end of the "light" axis became insignificant.

The locomotor activity rhythms of both species showed a rather crepuscular pattern (Fig. 3), with the greatest hourly percentage of activity occurring just before or just after onset of the light period, depending on the species. The large-

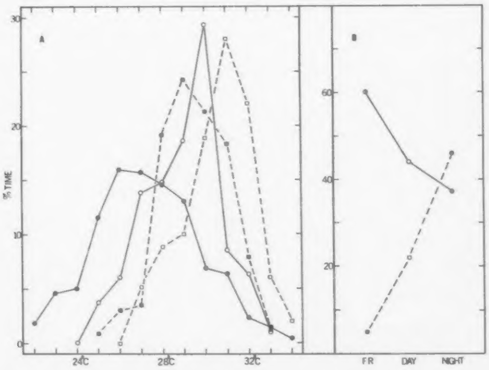

FIG. 2. Percentages of time spent by largemouth bass (*Micropterus salmoides*) (circles and solid lines) and smallmouth bass (*M. dolomieui*) (squares and dotted lines) A, at various temperatures and B, in the chambers which had the brightest light intensity (1000 lx) when the lights were on. Open symbols represent the normal "day" or lights-on period, closed symbols the normal "night" or lights-off period. Half-darkened symbols (FR) in B represent percent of time lights were on (1000 lx) when controlled by the fish, with no exogenous light cycle (free running). Each condition represents pooled data for five fish.

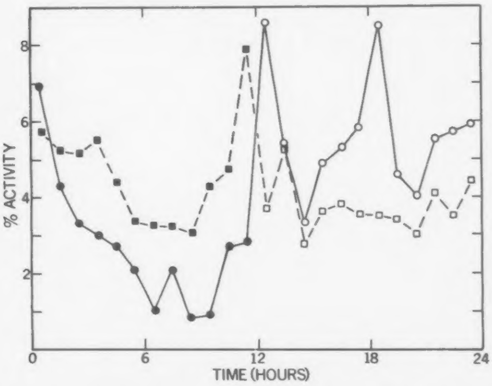

FIG. 3. Hourly percentages of total 24-h activity for largemouth (circles) and smallmouth (squares) basses, measured as total light-beam interruption events. Solid symbols represent normal hours of darkness ("night"), open symbols normal lights-on period ("day"). Pooled data for five fish of each species.

mouth bass showed greater activity during the light period than did the smallmouth bass, which showed a much greater degree of light avoidance. Other field and laboratory studies tend to confirm the preference of smallmouth bass for shadows, as well as the generally crepuscular nature of these predators (see Reynolds and Casterlin 1976b).

In another series of experiments, the bass were allowed to actively control the lights in an "on–off" manner along the light axis, while thermoregulating along the other axis. Light intensity was 1000 lx with the lights on and about 1 lx with the lights off. As expected, the smallmouth bass turned the lights on far less often (4% of the time) than did the largemouth bass (60%; Fig. 2B). The proportion of "lights-on" time bore no apparent relationship to time of day for either species, but the rate of on–off switching exhibited a characteristic crepuscular pattern similar to Fig. 3. This, along with other experiments in constant light conditions, indicated that the observed locomotor activity rhythms are endogenous (cf. Reynolds and Casterlin 1976b). This is further supported by investigations with another centrarchid, the bluegill (*Lepomis macrochirus*); these showed an endogenous diurnal locomotor activity rhythm which persisted under constant light or temperature conditions, or when either or both were controlled by the fish (Reynolds and Casterlin 1976c). The thermal preference rhythms and the locomotor activity rhythms seem to be endogenous in these centrarchid species, as they will persist in the absence of an exogenous cycle.

I have observed no indication of any significant interaction between the light and temperature responses, as similar thermoregulatory behavior occurred under different light regimes, either constant or endogenously or exogenously controlled. Similarly, light intensity preferences and locomotor activity rhythms (Reynolds and Casterlin 1976c) apparently do not differ significantly, whether the fish are actively thermoregulating or held at various fixed temperatures. In summary, evidence to date indicates no apparent interaction between light intensity and temperature responses along the two axes; responses to the two factors seem to be independent for the species studied.

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Differences in Swimming Performance Among Strains of Rainbow Trout (*Salmo gairdneri*)

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THOMAS, A. E., AND M. J. DONAHOO. 1977. Differences in swimming performance among strains of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 34: 304–306.

Swimming performance profiles, relating fish size to swimming time, were established for three strains of rainbow trout (*Salmo gairdneri*). No differences were found in slope of regressions; only in level at each size of fish. Swimming performances of New Zealand and Sand Creek strains did not differ, but were superior to the Manchester strain. In stamina results from

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189-day-old fish from individual matings of seven strains and various crosses, similar strains and crosses had closely matching profiles whereas profiles of unrelated groups were variable. Comparison of slowest, average, and fastest growing fish within the New Zealand strain showed that swimming ability was not related to growth rate.

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Nous avons déterminé les profils de performance de nage, faisant la relation entre la taille du poisson et le temps de nage, chez trois lignées de truite arc-en-ciel (*Salmo gairdneri*). Nous n'avons pas trouvé de différences dans les pentes des régressions; seulement dans les niveaux à chaque taille de poisson. Les performances de nage des lignées de Nouvelle-Zélande et du ruisseau Sand ne diffèrent pas, mais sont supérieures celles de la lignée de Manchester. Dans des tests d'endurance sur des poissons âgés de 189 jours provenant d'accouplements individuels de sept lignées et de divers croisements, les lignées et croisements semblables ont des profils qui se correspondent étroitement, alors que les profils de groupes non apparentés sont variables. Une comparaison des poissons de la lignée de Nouvelle-Zélande à rythmes de croissance lent, moyen et rapide démontre que la performance de nage n'est pas liée au rythme de croissance.

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SWIMMING performance of salmonids can be affected by factors such as disease, nutrition, stage of development, and rearing environment (Thomas et al. 1964; Fowler and Banks 1967; Thomas et al. 1969; Burrows 1964, 1969). Many authors have shown species differences in swimming performance between various salmon and trout, but differences between strains of the same species have rarely been considered. Usually only one strain is maintained at a location and comparisons would be confounded by stresses of transportation and differences in nutritional backgrounds and rearing environments. Apparent differences were noted by Thomas et al. (1964) between races of chinook salmon (*Oncorhynchus tshawytscha*) and by Green (1964) and Vincent (1960) between wild and domestic brook trout (*Salvelinus fontinalis*), but these groups had different environmental backgrounds. Differences between strains could be important in comparing results of different investigators, in understanding distribution among habitats within a species, and in designing fish passage and diversion equipment. Swimming performance of a number of strains of rainbow trout (*Salmo gairdneri*) has been investigated at the Fish Genetics Laboratory, Beulah, Wyoming, as one factor in the characterization of the strains. This report presents results of stamina determinations, (time that fish can maintain position in testing apparatus) on various strains under the same environmental and test conditions.

Materials and methods — Seven strains of rainbow trout maintained at the laboratory and eight crosses between strains were evaluated in a stamina tunnel. A strain in this study is defined as a breeding population that has been geographically and reproductively isolated from other such populations long enough to be recognized as having distinct characteristics in growth pattern and life history. The strains tested were Manchester from the Manchester (Iowa) National Fish Hatchery, Wytheville from the Wytheville (Virginia) National Fish Hatchery, University of Washington and Donaldson strains from Seattle, Washington, DeSmet from Lake DeSmet in Wyoming, Sand Creek from spring ponds on the Fish Genetics Laboratory grounds (Wyoming), and New Zealand, which originated from eggs sent to New Zealand from California in the 1890s. In addition to large populations of different strains formed by multiple matings, individual matings were made of strains and crosses of various strains throughout the August–May spawning season, and small populations of fish from these matings were maintained for various growth evaluations. Of the three most abundant strains tested, the Manchester is a fast-growing, domesticated, fall-spawning strain, and the New Zealand and Sand Creek strains are more streamlined, grow slower, have had shorter periods of domestication, and spawn in the spring.

Fish of the Manchester, New Zealand, and Sand Creek strains were maintained in tanks 2 m diam and 0.3 m deep. Samples of 150 average-sized fish were taken from each strain population at 2-wk intervals throughout the rearing season and selected at random into three 50-fish groups for stamina testing. A number of 50-fish groups of the smallest and largest fish of the same age within the New Zealand population were compared with fish of average size. The groups were unfed and kept in trough compartments for at least 18 h before being tested (without change in water temperature) in the stamina tunnel. Groups varied from 8.7 to 28.5 g avg. wt. Larger fish were not used due to suspected effects of crowding in the rearing tanks.

Water temperature during all rearing and testing was 12°C. The standard, open-formula trout diet (PR-6) used in Service hatcheries was fed to all groups with feeding frequencies and particle sizes determined by routine hatchery procedures.

Stamina of fish populations maintained from single matings — both pure strains and crosses — was tested under the same conditions except that tests were done only at 189 days of age and on two, rather than three, 50-fish groups.

A commercial model of the stamina tunnel, utilizing a recirculating, closed water system, described by Thomas et al. (1964) was used for all testing. Groups of fish placed in a Plexiglas® tube, 30 cm diam and 183 cm long, swam against a flow controlled by the differential in water level between the head and tail boxes. Flow rate was adjustable with a varidrive pulley and axial flow pump. Water temperature and oxygen content remained constant during testing. The testing procedure included 10-min orientation at 13.7 cm/s water velocity and stepwise increases of 3.7 cm/s each min until all fish were flushed from the tunnel. An electric field at the lower end of the tube induced the fish to continue swimming until exhausted. Exhausted fish were swept from the tube, tallied at 1-min intervals, and weighed at the completion of the test.

Average swimming time of each group was used to develop performance profiles (linear regressions). A stamina rating allowing direct comparisons between fish of different sizes has been developed using the formula $\mathrm{Pe} = a + b\sqrt[3]{w}$, where Pe represents expected performance, a the value of the performance when the weight is 0, b the increase in performance for each cube weight gain, and $\sqrt[3]{w}$ the average cube root of the group. Performances were adjusted according to stamina ratings of the Manchester strain, which was assigned an expected performance rating (Pe) of 100. Ratings of individual fish groups were based on the formula 100 (Po)/Pe, where Po is the observed performance of that group.

Results and discussion — Performance of different strains — Performance profiles were expressed as linear regressions (Fig. 1A) and represent results from 1500 fish (30 groups) for the Manchester strain, 1250 fish (25 groups) for the Sand Creek strain, and 1650 fish (33 groups) for the New Zealand strain. In this manner, the expected performance (swimming time) of a specific-sized fish (cube root) could be determined for each strain.

All regressions in Fig. 1A were highly significant. Analysis of covariance revealed these relations: 1) slopes of the three profiles were not significantly different, 2) differences in levels of performance of Manchester and New Zealand strains and of Manchester and Sand Creek strains of any given size were highly significant, and 3) performance of New Zealand and Sand Creek strains was not significantly different. The swimming ability of the Manchester strain was less than that of the other strains for all sizes of fish tested.

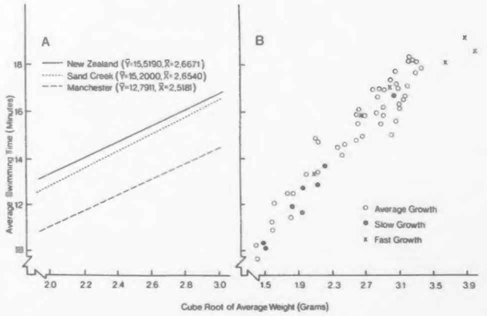

FIG. 1. A, Performance profiles for three strains of rainbow trout (*Salmo gairdneri*); B, Swimming performances of slow-, average-, and fast-growing fish from the New Zealand strain. All fish were the same age.

For approximately 210 groups stamina results were compared with performance profiles for the Manchester, New Zealand, and Sand Creek strains shown in Fig. 1A. Manchester, Wytheville, or Manchester–Wytheville crosses closely fitted the Machester performance profile, whereas New Zealand and Sand Creek strains, and crosses of the two, closely fitted the New Zealand and Sand Creek profiles. Groups which were Donaldson–DeSmet, DeSmet–Sand Creek, Donaldson–University of Washington, and Donaldson–DeSmet–University of Washington crosses also fitted the New Zealand and Sand Creek profiles. A few groups of University of Washington and DeSmet–Sand Creek–Donaldson strains had intermediate performance between the Manchester and New Zealand profiles. Fifteen tests of groups from Sand Creek–Donaldson crosses resulted in scattered performances; some were near each profile and some between them. Other types of crosses either did not survive to the 189-day evaluation period or could not be made due to differences in spawning time.

In addition to velocity profiles, we compared stamina ratings for 15.6-g fish of the Manchester, New Zealand, and Sand Creek strains, using the Manchester performance profile as a base (15.6 g was the average weight of the fish used to develop all three profiles). Stamina ratings were 100.0, 117.6, and 115.0 for the Manchester New Zealand and Sand Creek strains, respectively. Average swimming time values for the fish were 12.7, 15.0, and 14.6, respectively.

Effect of growth rate on performance — The rapidly growing Manchester strain had lower swimming ability than the slower growing New Zealand and Sand Creek strains. To determine whether swimming ability for these strains was due to growth rate rather than genetic differences, we compared the fastest and slowest growing fish within the New Zealand population (of identical age) with fish of average growth rate (Fig. 1B). No major variation from that of average-sized fish was apparent.

The performance profiles show that the streamlined, semiwild strains (New Zealand and Sand Creek) had better relative swimming ability than the deeper-bodied, domesticated Manchester strain. Such information should be useful in fishery management. The better-swimming strains could be stocked in stream environments where swimming ability would be important to survival and the faster-growing Manchester strain could be restricted to lakes and "put-and-take" fisheries where rapid growth is of major importance. The slope of the Sand Creek profile varied somewhat from the other two (not significantly), and was probably influenced by fewer small-sized fish being tested.

Results from the 189-day individual groups confirmed the reliability of the performance profiles. Since the fastest and slowest growing fish in the New Zealand strain had results that did not vary from their performance profile, we concluded that differences in swimming ability between strains were not related to growth rate.

The performance ratings presented for 15.6-g fish should be valid for any sized fish since there was no significant difference in the slope of the performance profiles. The mean fish weight of the three strains was used to compare the performance ratings with the average swimming time values. The actual swimming time values would vary depending upon fish size but the relationship between the three strains should remain constant.

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BOOK REVIEWS/COMPTES RENDUS

Physical and Chemical Characteristics of Aquatic Humus. *E. T. Gjessing. Ann Arbor Science Publishers, Ann Arbor, Michigan, 1976. vii + 120 p. $14.50.*

Three different perspectives can be chosen to understand humus, humic substances, and the like. While each one does not exclude the other, it conditions experimental choices, and qualifies data interpretations.

They may be epitomized as follows. a) The physical and chemical approach: humus is analyzed and classified according to the requirements of these disciplines. As a general rule, the emphasis is on structures and reactivities common to humic substances from quite different sources. b) The ecological standpoint: the study of the physicochemical attributes of humus is subordinated to the appreciation of its active and passive relationships with the communities of different ecosystems, and its effect on the quality of the environment. In this instance, the trend is to display spatial and temporal diversifications. c) The technological, or applied approach: this one is taken by water work engineers and agronomists. It is also the leitmotif of the author. Therefore, mission-oriented objectives pervade the book. However, physical and chemical subjects are given adequate treatment, justifying the title.

The book summarizes mainly the work done on aquatic humus at the Norwegian Institute for Water Research. A fairly large critical review of the literature dealing with the work performed on humus in several other Institutes is given as well. The latter undertaking was an effort to keep the NIVA abreast of the advances in the field. Soil science, with some emphasis on publications from eastern European countries, plays a prominent role as an information source. Some theoretical inconsistencies in data interpretation have been pointed out, and some operational compromises have been accepted as substitutes. For example, in discussing laboratory findings concerning the humus capability of sequestering several polyvalent cations, it is pointed out that the fundamental thermodynamic parameter, the stability constant, can not be measured because the molecular weight of the ligand(s) is not known. Therefore, the theoretically useful predictive application of the data gained from these experiments is jeopardized.

The experimental work encompasses several approaches. Some was done in the laboratory, trying to maximize the quality of the methods utilized and the information concerning the physical and chemical properties of aquatic humus provided. Various techniques, widely used in biophysical chemistry, like gel chromatography, ultrafiltration through graded porosity membranes, and electrofusing, have been utilized extensively on a performance-oriented basis. Some work involved observations of the effects of time-honored technologies on aquatic humus, and suggested some modification in pilot-plant experiments. Finally some was done in natural habitats, evaluating the responses of the colored organic compounds of lentic and lotic waters to natural physical and chemical factors, and comparing these evaluations with laboratory experimental results.

The bleaching effect on color, and photooxidation of dissolved organic carbon induced by ultraviolet irradiation at wavelengths shorter than 240 nm are well known. The author's approach to this topic may be chosen as an example of his general trend in data acquisition. Partial decrease of color, organic carbon, and iron concentrations has been observed in laboratory experiments using stream-simulating apparatus. Part of the system was irradiated with ultraviolet radiation, which was found to enhance the effects. Under natural conditions, differentiated decrease of the same parameters were observed in lakes utilized in water works, or in brooks originating from a peaty area. Since the solar radiation reaching the earth surface does not contain appreciable radiation of wavelengths shorter than 290 nm, the possibility, suggested by the author, that ultraviolet photooxidation is effective in natural habitats must be ruled out. Rather, the effects observed in natural environments can be explained by suggested alternative mechanisms, including degradation, or destabilization of humic micelles. Incidentally, microbial effects on laboratory experiments lasting a long time, even years, should be taken into account so that real causal agents can be recognized. From the analysis of the data offered by the author, it appears to me that a very important factor is downstream dilution with less colored waters.

The micellar destabilization, i.e. poorly understood physical and chemical changes of the aquatic humus, may be due to biogenic and/or abiogenic causes. They become apparent through structural rearrangements, coupled to changes in solubilities, partition coefficients between water and sedimentary materials, and behavior during gel chromatography. Bleaching effects of unexplained origin can not be dismissed.

The vast amount of freshwaters loaded with colored organic substances justifies the author's concern with the removal of organic matter from water works, and from sewage effluents before they are reintroduced into continental and oceanic water bodies. Humic waters are widespread in many regions of the earth besides Norway. To name but a few: many lakes in Finland, thoroughly studied for a long time by Ryhänen and his colleagues; several lakes of the Canadian Precambrian Shield; and the Congo river with its tributaries, where along with the exceedingly low mineral content, humus has been tentatively coupled to the spreading of water hyacinth. After effluents are processed, the final product may be water still containing organic compounds without color and other objectionable organoleptic properties, yet with unknown biological properties. This must be avoided: seemingly the author suggests that this can be achieved if 100% mineralization is assured. In keeping with the title of the book, the author deals only passingly with the biological quality of the water before and after processing. Problems connected with its inorganic, microbiological, and viral properties are not discussed. Those connected with photosynthetic or secondary productivity, important during storage in reservoirs, are also neglected. I like to recall them because they are fundamental for proper management of water resources.

The dominant objectives have been approached by summarizing trends in aquatic and soil organic chemistry, presenting some plausible problems, and discussing or offering solutions. Objective constraints in the whole issue of humus make it difficult to agree or disagree with remarks and solutions. One should realize that a large time lag intervenes before new acquisitions in chemistry and physical chemistry can be applied to environmental issues.

If no encyclopedic coverage, nor breakthroughs in aquatic humus related problems are expected, the book should be useful to water work engineers and to agronomists as an introductory compendium of numerous problems they likely will be faced with. Ecologists dealing with the properties of humus will find interesting information in it, although some questionable, for example when the author claims that aquatic humus is the water soluble portion of soil humus, and corresponds to the fulvic acid fraction of the latter. Accordingly, the reader should have some familiarity with the physical and chemical properties of humus, and of natural polymers as well. He should also have some knowledge of environmental problems, when humus physical chemistry has to be coupled to the study of ecological issues.

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(R112)

Gas Bubble Disease. Proceedings of a Workshop held at Richland, Washington, October 8–9, 1974. *Edited by D. H. Fickeisen and M. J. Schneider. National Technical Information Service, U.S. Department of Commerce, Springfield, Virginia. 1976. viii + 123 p. $6.00 U.S.; $8.50 foreign.*

Water can become supersaturated with air in several ways: most common is the impaction of falling water on standing water that frequently occurs under waterfalls and particularly in hydroelectric developments. Ground water can become supersaturated by temperature and pressure fluctuations. Aquarium systems frequently develop supersaturation if air finds its way into pumps through leaky suction lines or wave action at intakes.

The effects on fish are dramatic: eyes bulge grotesquely, membranes of mouth and fins strain to bursting by accumulated air. It can end in convulsions, lethargy and death, or in blinded or crippled survivors. Fish apparently cannot detect this lethal condition. Concern has been building for years over the often spectacular economic losses that occur when migrating salmonids and hydroelectric developments meet.

What literature has appeared to date has been mainly anecdotal. Notably scarce have been studies on pathogenesis, measurement, and amelioration methods. The symposium collection *Gas Bubble Disease* is one of the first collections, certainly the first worthwhile collection, of data on the occurrence and measurement of supersaturation and on the pathogenesis of gas bubble disease.

Migrating salmonids naturally occupy the attention of most of the contributors, but invertebrates and resident fish species have not been neglected. No macroscopic species, even daphnia, seems to be immune to gas bubble disease, although the more active species tend to succumb to lower levels, and more precipitously (often following vigorous activity). The volume consistently reports quantitative measurements of supersaturation, allowing a reader to develop a feel for the combinations of supersaturation and exposure times that precipitate the disease.

The work on pathogenesis includes the effects of sublethal levels on blood chemistry, lateral line function, and behavior patterns. At lethal levels, peripheral hemostasis appears to be the immediate cause of death.

Measurement technique is not treated in an integrated fashion, but many individual articles go into some detail on this subject. A thorough reading of the methods sections will provide a background for this very tricky aspect of the study of gas bubble disease.

Unfortunately missing is significant reference to water treatment methods for alleviating supersaturation. This, I think, represents a gap in the research being done, rather than a deficiency of this particular book.

The collection ends with a series of summary statements, or "Round Table Discussions." These should be read first if one is attempting to tackle the book as a whole; they are essentially abstracts of the contained papers, with suggestions for future directions of research.

Gas Bubble Disease cannot be rated against similar works, being the only work of its kind. Considering the unavoidable deficiencies of the symposium format (including the 18-month publication delay), give it an eight on a scale of ten, a very worthwhile addition to the aquatic biologist's library.

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(R115)

The Fishes of Missouri. *William L. Pflieger. Missouri Department of Conservation, 1975. viii + 343 p. $7.50 soft cover, $10.00 hard cover.*

I collect books on regional fish faunas, so I was glad to see another one come from the rich ichthyology of the midwest. Nearly every state of this region has either a popular book or technical publications on the identification and distribution of the fishes within its boundaries. I thought that such books had explored nearly every possible way of presenting a technical subject that would be useful to both the biologist and the public. As a result, I was surprised and pleased at the refreshing presentation in this book.

To me, the strength of the book lies in the use of the drawings and in its organization, and these two attributes will be stressed in these remarks. Every family and every species is illustrated by line drawings printed on a colored background. Drawings show every important key character. For example, the couplets of the key are located on the left-hand side of the page, and the pair of drawings illustrating each member of the couplet is on the right side. For the most part the drawings are not executed in extraordinary detail; nevertheless, the artist captured the image of the species so that it can be identified at a glance. The key features of each drawing are labeled. Since the fauna is comprised of nearly 200 species, the artist and the author worked many hours together portraying each one to their satisfaction.

Following the detailed key to the species of each family is a short discussion of the species which includes the key characters, life colors, size, meaning of the scientific name, distribution in the state (map with spot localities and colored range), distribution in North America (map), habitat, and finally some interesting notes about its habits, life history, and importance. The text is written in simple, straight-forward language and the editors have allowed the author considerable freedom to expand those sections he feels will be of interest to the reader. For example, the habits and life history of the creek chub take up nearly a whole, double-columned, 20 × 25-cm page.

The first 20 pages of the book are devoted to general topics designed to interest the reader in the variety of species, the variety of habitats in Missouri, and how to collect, identify, and observe fishes. A section on "Structure and Life Ways" includes many facts of interest to the layman, but anyone tackling such a broad subject in such abbreviated fashion is bound to fall short. This section, of all I read, could have been handled better.

Sixteen pages are devoted to color photographs. For the most part they are good reproductions, but some habitat shots are too dark to be attractive. The book closes with a glossary, references, and index. The references are presented by number in the text and in the reference list. This is not my preference in this regard since the reader is forced to run through the complete bibliography to find a reference in which he is interested.

For the fisheries biologist and systematist this book is an invaluable resource, and my guess is that the layman will find it more useful than most other state reports. Dr Pflieger and the Missouri Department of Conservation are to be congratulated for producing a first-class book on the fishes of their state.

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(R120)

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Pleistocene watershed exchanges and the fish fauna of the Peel River basin, Yukon Territory **R. A. Bodaly and C. C. Lindsey**
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Estimates of tag extrusion and initial tagging mortality in Atlantic herring (*Clupea harengus harengus*) released with abdominally inserted magnetic tags **G. H. Winters**
Hydrocarbons in eastern Canadian marine waters determined by fluorescence spectroscopy and gas–liquid chromatography **P. D. Keizer, D. C. Gordon Jr., and J. Dale**
Temperature and photoperiod regulation of rheotropic behavior in prespawning common shiners, *Notropis cornutus* **J. J. Dodson and J. C. Young**
Statistical assessment of the age–length key **D. K. Kimura**
Host and seasonal associations of *Echinorhynchus salmonis* (*Acanthocephala*: *Echinorhynchidae*) in Lake Michigan fishes **O. M. Amin and J. M. Burrows**
Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica* **B. D. Taubert and D. W. Coble**
Validity of the subspecies designation for the dwarf white sucker (*Catostomus commersoni utawana*) **R. J. Beamish and E. J. Crossman**
Modèles linéaires de prédiction des débarquements de homard aux Iles-de-la-Madeleine (Golfe du Saint-Laurent) **F.-R. Boudreault, J.-N. Dupont et C. Sylvain**
Isolation and characterization of an anaerobic bacterium, *Eubacterium tarantellus* sp.n, associated with striped mullet (*Mugil cephalus*) mortality in Biscayne Bay, Florida **L. R. Udey, E. Young, and B. Sallman**
Filamentous algae as a food source for the brown bullhead (*Ictalurus nebulosus*) **J. M. Gunn, S. U. Qadri, and D. C. Mortimer**

Notes

Detection of trace contaminant effects in aquatic ecosystems **S. N. Luoma**
Production of the euphausiid crustacean *Thysanoessa raschii* in the Gulf of St. Lawrence **F. Berkes**
A red tide caused by the marine ciliate *Mesodinium rubrum* in Passamaquoddy Bay, including pigment and ultrastructure studies of the endosymbiont **A. W. White, R. G. Sheath, and J. A. Hellebust**
Resting eggs in *Pontella meadi* (Copepoda: Calanoida) **G. D. Grice and V. R. Gibson**
Nitrogen fixation (acetylene reduction) associated with sea urchins (*Strongylocentrotus droebachiensis*) feeding on seaweeds and eelgrass **M. L. Guerinot, W. Fong, and D. G. Patriquin**
Holding of mackerel (*Scomber scombrus*) in refrigerated sea water **D. W. Lemon and L. W. Regier**
Effects of chemical additives on extending the shelf life of frozen minced sucker (Catostomidae) flesh **D. G. Iredale and R. K. York**
Therapeutic treatment for epibiotic fouling on Dungeness crab (*Cancer magister*) larvae reared in the laboratory **W. S. Fisher and R. T. Nelson**
A relationship between two stock–recruitment curves **L. L. Eberhardt**
Survival of two bacterial fish pathogens (*Aeromonas salmonicida* and the enteric redmouth bacterium) in ozonated, chlorinated, and untreated waters **G. A. Wedemeyer and N. C. Nelson**

RECENT PUBLICATIONS / RÉCENTES PUBLICATIONS

The number following each title in each series refers to the establishments listed at the end of the section.

Les nombres qui suivent les titres de chacune des séries désignent les établissements suivants à la fin de la section.

Recent Studies

Until 1970, reprints of these articles had been included in an annual volume of "Studies," composed of two parts. The final binding was the 1969 Studies, Part 2, which included No. 1355. These are no longer bound and distributed. We continue, however, to number and list all articles on investigations published in other periodicals, which henceforth may be obtained in reprint form only from the author or originating establishment.

Récentes études

Jusqu'en 1970, ces articles étaient réimprimés, puis reliés dans un volume annuel d'études, comprenant deux parties. Les études de 1969, partie 2, incluant le n° 1355, ont été les dernières à être reliées. Nous n'en faisons désormais plus la reliure ni la distribution. Nous continuons cependant de numéroter les articles et d'en établir des listes d'études sur des recherches mentionnées dans d'autres périodiques. On peut donc se les procurer, sous leur forme réimprimée seulement, de l'auteur ou du service dépositaire.

2244. Smith, J. C. 1976. Body weight and the activities of pyruvate kinase, glucose-6-phosphatase and lactate dehydrogenase in the liver of the American plaice, *Hippoglossoides platessoides*. Comp. Biochem. Physiol. 53B: 277–282. (7)

2245. Payne, J. F., and A. K. Bal. 1976. Cytological detection of poly(ADP-ribose) polymerase. Exp. Cell Res. 99: 428–432. (9)

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2251. Sutterlin, A. M., and S. Waddy. 1976. Tentacle movement patterns involved in feeding behaviour of the sea cucumber, *Cucumaria frondosa*. Mar. Behav. Physiol. 4: 17–24. (6)

2252. Mason, J. C. 1974. Crayfish production in a small woodland stream. Proc. 2nd Int. Crayfish Symp. 1974. Louisiana. 449–479. (1)

2253. Gilgan, M. W., and B. G. Burns. 1976. The anesthesia of the lobster (*Homarus americanus*) by isobutanol injection. Can. J. Zool. 54: 1231–1234. (8)

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2256. Parsons, L. S., A. T. Pinhorn, and D. G. Parsons. 1976. An evaluation of the northern Newfoundland–Labrador and Flemish Cap redfish fisheries. ICNAF Res. Bull. 12: 37–48. (9)

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2258. Pitt, T. K. 1976. Food of yellowtail flounder on the Grand Bank and a comparison with American plaice. ICNAF Res. Bull. 12: 23–27. (9)

2259. Manzer, J. I. 1976. Distribution, food, and feeding of the threespine stickleback, *Gasterosteus aculeatus*, in Great Central Lake, Vancouver Island, with comments on competition for food with juvenile sockeye salmon, *Oncorhynchus nerka*. Fish. Bull. 74: 647–668. (1)

2260. Laskowski, M. et al. 1976. Nucleolytic enzymes of the venom of *Crotalus adamanteus*. Anim. Plant Microb. Toxins 1: 235–248. (2)

2261. Hurst, E. E. 1976. Dimethylnitrosamine levels in untreated herring meals. J. Sci. Food Agric. 27: 600–602. (2)

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Manuscript Reports

This series includes unpublished preliminary reports and data records not intended for general distribution. They should not be referred to in publications without clearance from the issuing establishment and without clear indication of their manuscript status.

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342. Schindler, D. W. 1976. The impact statement Boondoggle. Science 192: 509. (4)
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The following translations have been prepared by or for the Fisheries and Marine Service, for use in its investigations. Copies are available from Translation Services, Canada Institute for Scientific and Technical Information, National Research Council of Canada, Ottawa, Ontario K1A 0S2, at a cost of 22¢ per page and a minimum charge of $2.20. Information on translations can be obtained from the Scientific Information and Publications Branch.

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ANNOUNCEMENTS / AVIS

Fourth International Congress of Parasitology (ICOPA IV)

The Congress will be held from August 19 to 26, 1978 in the Palace of Culture and Science, Warsaw, Poland.

The scientific program will consist of two parts. First, short communications, published in advance of the meetings, will be discussed at respective Sections grouped in one of the eight Divisions. These will replace the formal presentation of full length individual papers. Second, there will be a series of Plenary Review Sessions closely corresponding to the respective Divisions.

Scientists interested in receiving further information and a preregistration form are requested to contact the Secretary General of ICOPA IV — Professor Bernard Bezubik, Department of Parasitology, University of Warszawa, 00-927 Warszawa, Poland.

Quatrième Congrès international de parasitologie (ICOPA IV)

Le congrès se tiendra au Palais de la culture et de la science, Varsovie, Pologne, du 19 au 26 août 1978.

Le programme scientifique comprendra deux parties. En premier lieu, de brèves communications, publiées à l'avance, seront l'objet de discussions au sein de sections respectives groupées en une de huit divisions. Ces communications remplaceront la présentation formelle de travaux individuels plus complets. En second lieu, il y aura une série de séances de revue plénaires correspondant étroitement aux divisions respectives.

Les scientifiques désirant plus de renseignements et une fiche de préenregistrement sont priés de s'adresser au Secrétaire-général de ICOPA IV — M. le Professeur Bernard Bezubik, Département de parasitologie, Université de Warszawa, 00-927 Warszawa, Pologne.

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Volume 34, No. 2, February 1977

Volume 34, n° 2, février 1977

Editorial/Éditorial **J. C. Stevenson** 173–176

Uptake from sea water and clearance of ^{14}C-*p,p'*-DDT by the marine copepod *Calanus finmarchicus* **G. C. H. Harding and W. P. Vass** 177–182

Population dynamics and marine mammal management policies **L. L. Eberhardt and D. B. Siniff** 183–190

Host–parasite relationships between sockeye salmon, *Oncorhynchus nerka*, and *Salmincola californiensis* (Copepoda: Lernaeopodidae) **Z. Kabata and B. Cousens** 191–202

Immunization of Pacific salmon: comparison of intraperitoneal injection and hyperosmotic infiltration of *Vibrio anguillarum* and *Aeromonas salmonicida* bacterins **R. Antipa and D. F. Amend** 203–208

Chemical and sensory changes in round inshore male capelin (*Mallotus villosus*) during prolonged storage for 24 months at −23°C **D. H. Shaw and J. R. Botta** 209–214

Heinz body hemolytic anemia in coho salmon (*Oncorhynchus kisutch*) exposed to chlorinated wastewater **J. A. Buckley** 215–224

Surface area of the euphausiid *Thysanöessa raschii* and its relation to body length, weight, and respiration **G. C. H. Harding** 225–231

Changes in the size structure of cichlid populations of Lake Malaŵi resulting from bottom trawling **J. L. Turner** 232–238

Preferred, avoided, and lethal temperatures of fish during rising temperature conditions **D. S. Cherry, K. L. Dickson, J. Cairns Jr., and J. R. Stauffer** 239–246

Life histories of *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories **D. M. Rosenberg, A. P. Wiens, and O. A. Sæther** 247–253

Responses to crude oil contamination by *Cricotopus* (*Cricotopus*) *bicinctus* and *C.* (*C.*) *mackenziensis* (Diptera: Chironomidae) in the Fort Simpson area, Northwest Territories **D. M. Rosenberg, A. P. Wiens, and O. A. Sæther** 254–261

Notes

Egg and larval survival of white suckers (*Catostomus commersoni*) at low pH **J. R. Trojnar** 262–266

Rapid chromosome preparations from solid tissues of fishes **A. D. Kligerman and S. E. Bloom** 266–269

Nutritional implications of surface feeding by gizzard shad (*Dorosoma cepedianum*) on insect remains in an Ohio reservoir **P. A. King, T. E. Wissing, E. J. DeVillez, M. J. Chimney, and W. B. Randall Jr.** 269–273

Fecundity of *Balanus balanoides* in Passamaquoddy Bay **D. C. Arnold** 273–275

Kinetics of lampricide (TFM, 3-trifluoromethyl-4-nitrophenol) residues in model stream communities **A. W. Maki and H. E. Johnson** 276–281

Occurrence of piscine erythrocytic necrosis (PEN) in the blood of the anadromous alewife, *Alosa pseudoharengus*, from Maine coastal streams **S. W. Sherburne** 281–286

Comparison of an ecological model of Lake Ontario and phosphorus loading models **D. Scavia and S. C. Chapra** 286–290

Factors in interpreting data obtained by diel sampling of fish stomachs **D. M. Eggers** 290–294

Sex ratio of American eels, *Anguilla rostrata*, from the Matamek River system, Quebec, with remarks on problems in sexual identification **J. A. Dolan and G. Power** 294–299

Fish orientation behavior: an electronic device for studying simultaneous responses to two variables **W. W. Reynolds** 300–304

Differences in swimming performances among strains of rainbow trout (*Salmo gairdneri*) **A. E. Thomas and M. J. Donahoo** 304–307

Book Reviews/Comptes rendus: Physical and chemical characteristics of aquatic humus (E. T. Gjessing), **D. V. Povoledo**; Gas bubble disease. Proceedings of a workshop held at Richland, Washington, October 8–9, 1974 (D. H. Fickeisen [ed.]), **W. R. Penrose**; The fishes of Missouri (W. L. Pflieger), **S. D. Gerking** 308–310

Coming next month/À paraître le mois prochain 311

Recent publications/Récentes publications 312-315

Announcements/Avis *Inside back cover*

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